RESEARCH ON THE EPIDEMIOLOGY AND PREVENTION OF HIV IN RURAL SOUTH WEST UGANDA, 1989-2010

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Declaration

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Abstract

This thesis is based on research on the epidemiology and prevention of HIV among adults in rural Masaka district, Uganda (1989-2010). Arising from this research are 10 published papers, which I have used to explore three research questions: (i) what are the trends in HIV prevalence and incidence in rural Uganda? (ii) what are the key determinants of these trends? (iii) what new strategies could be used to prevent HIV infection in this population?

The studies involved four adult cohorts: a general population cohort to monitor HIV prevalence and incidence trends through annual household and serological surveys; an STD/behavioural intervention cohort; a cohort for HIV vaccine preparedness studies; and a cohort of HIV-negative women in discordant couple relationships to evaluate HIV biomedical interventions.

The findings from the published papers are summarised. Additional analyses were conducted to include prevalence and incidence data up to 2008. The time trends were examined using a proximate-determinants framework. A comparison of the observed trends was also made with other available national data as well as data from two other African countries.

There was a significant decline in HIV prevalence for all ages in the 1990s followed by an increase in the 2000s. Similarly, HIV incidence declined significantly in the 1990s in all adults although there were no clear trends in the 2000s. A net outflow of HIV positive migrants, mortality among HIV positive individuals and a decrease in risky sexual behaviour in the 1990s seem to have been important factors in explaining the decline. The increase in prevalence in the 2000s is explained partly by improved survival due to ART and possibly complacency leading to increased risky sexual behaviour.

Two HIV intervention trials (of STD/behaviour change and a phase 3 vaginal microbicide) are also discussed. Neither trial showed any effect on HIV transmission. To explain the findings from these trials, a conceptual framework for discussing negative results from HIV prevention trials has been used.. The possible explanation for the negative results was that the concepts were right but the actual interventions were "inert" or insufficient to demonstrate impact. There was also evidence that there were deficiencies in the design and delivery of the school-based component of the behavioural intervention. In recognition that the epidemic continues unabated, I have addressed priorities for future HIV prevention research on biomedical and behavioural interventions.

The cohorts established and research capacity built will continue to provide new opportunities for monitoring and preventing the transmission of HIV in this population.

List of Abbreviations

ABC	Abstinence, Be faithful, Condom use
ACP	AIDS Control Programme
AE	Adverse event
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal clinic
ART	Antiretroviral therapy
CDC	Centers for Disease Control
CRT	Cluster randomized trials
DAIDS	Division of AIDS
DHS	Demographic and Health Survey
HIV	Human immunodeficiency virus
HR	Hazard ratio
HSV-2	*Herpes simplex* virus type-2
IEC	Information Education Communication
KABP	Knowledge attitude behaviour practice
MDP	Microbicides Development Programme
MRC/UVRI	Medical Research Council/Uganda Virus Research Institute
MSM	Men who have sex with men
RR	Rate ratio
PMTCT	Prevention of mother-to-child transmission
PrEP	Pre-Exposure Prophylaxis
Pyrs	Person years
RCT	Randomized controlled trial
RR	Rate ratio
SSA	Sub Saharan Africa
STD	Sexually transmitted disease
STI	Sexually transmitted infection
VCT	Voluntary counselling and testing
WHO	World Health Organization

Chapter 1. Introduction

Summary

This chapter provides a background to the epidemiology of HIV in Uganda, based on research conducted in cohorts of the rural adult population in SW Uganda. In this chapter, I describe the overall research programme, its development, and main findings from this research on: (i) trends in, and risk factors for HIV infection; (ii) the impact on HIV incidence in a sexually transmitted disease (STD)/behavioural interventions trial and (iii) the impact on HIV incidence in a phase III vaginal microbicide efficacy trial. I describe the key determinants of HIV incidence and prevalence using a proximate-determinants framework, and also explain which of these have been addressed by the various studies in this thesis. I played a key role in setting up and developing these studies, directing the epidemiological research and implementing the trials. I also present the objectives of this thesis and the papers that were published in peer-reviewed journals between 2000 and 2010.

1.1 Background

After more than two decades, the global spread of HIV continues, with approximately 2.6 million new infections in 2009(1). Over 90% of new infections occur in low income countries, with the majority occurring in sub-Saharan Africa (SSA). In SSA an estimated 1.8 million people became infected in 2009. HIV has had a large impact on economic growth and the health sector in heavily affected countries(2-4). However significant scientific knowledge has been gained to date that has helped us understand the determinants of HIV transmission, trends in HIV infection, the socio-demographic impact, and virological and immunological characteristics of the virus.

Though the spread of HIV infection in SSA has been shown to occur predominantly through heterosexual transmission, African studies reveal substantial between-country and within-country heterogeneity in HIV prevalence and incidence rates(5-7), and the

occurrence of a wide variety of HIV sub-types. The most predominant HIV-1 subtypes in Africa are A, C and D, of which subtypes A and D account for most (over 95%) of the infections in Uganda(8). The HIV subtype in East and South Africa is type-1 and the use of the term HIV in this thesis thereafter I refer to HIV-1.

From the late 1980s, several systems were set up in Uganda to monitor the HIV epidemic. These included the establishment of population-based epidemiological studies to determine HIV risk factors, the prevalence and incidence of infection, epidemiological trends, as well as the impact on mortality and fertility(9-10). In addition to these studies, the HIV epidemic has been monitored through a Ministry of Health sentinel surveillance system using antenatal and STD clinic attendees, and through periodic national sero-behavioural surveys. Both the epidemiological surveys and the national surveys have provided valuable data for monitoring trends as well as for planning and policy formulation in Uganda.

There has been some success in controlling the HIV epidemic in some countries in SSA, including Uganda which was one of the first African countries to report HIV infection in the early 1980s(11-13). The success of HIV control in Uganda, as evidenced by a fall in prevalence and incidence rates throughout the 1990s, has been attributed to a range of public health interventions including the establishment of the National AIDS Control Programme by the Ministry of Health, which implemented wide-scale health education (based on the "Abstinence, Be faithful, and Condom use (ABC)" strategy), HIV voluntary counselling and testing (VCT), and treatment of STDs(11). Despite these interventions HIV prevalence and incidence rates remain high and the decline in HIV prevalence observed in the mid and late 1990s has stopped. There is recent evidence indicating a levelling off of infection rates, and a possible increase in certain age groups(14).

1.2. The Mission of Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) Research Unit on AIDS

The MRC/UVRI Unit was established in 1989 in response to the rapidly expanding epidemic of HIV/AIDS in Uganda. The mission of the Unit is: (i) to investigate and document the determinants of HIV transmission and subsequent disease progression in an African context, (ii) to develop and evaluate effective interventions and (iii) to formulate new strategies for preventing HIV transmission and alleviating its medical and social consequences. The research findings are intended to inform health policy in Uganda and elsewhere, and contribute to the translation of research into policy and practice both locally and internationally.

1.3. Objective of this thesis

The overall objective of this thesis is to describe and critically examine research on the epidemiology and control of HIV infection in rural Masaka district, South-West Uganda from 1989 to 2010. The thesis explores three related research questions which have implications for the prevention of HIV transmission in African populations, based on the mission of the MRC/UVRI described above.

i. What are the trends in the epidemiology of HIV in Uganda?
ii. What are the key determinants of these trends?
iii. What new strategies could be used to prevent HIV infection in this population?

The research I will examine is based on work I conducted using rural population-based cohorts in Masaka district and comprises 10 papers published in peer-reviewed journals between 1999 and 2010.

1.4. The papers submitted as part of this thesis

Paper 1. **Kamali A**, Carpenter LM, Whitworth JAG, Pool R, Ruberantwari A, Ojwiya A. Seven–year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda. *AIDS* 2000; 14:427-434.

Paper 2. Mbulaiteye SM, Mahe C, Whitworth JAG, Ruberwantari A, Nakiyingi JS, Ojwiya A, **Kamali A.** Declining HIV-1 incidence and associated prevalence over 10 years in a rural population in south-west Uganda: a cohort study. *Lancet* 2002; 360:41-46.

Paper 3. Whitworth JAG, Mahe C, Mbulaiteye SM, Nakiyingi J, Ruberantwari A, Ojwiya A, **Kamali A.** HIV-1 epidemic trends in rural south-west Uganda over a 10-year period. *Tropical Medicine and International Health* 2002; 7:1047-1052.

Paper 4. Shafer LA, Biraro S, Nakiyingi-Miiro J, **Kamali A**, Ssematimba D, Ouma J, Ojwiya A, Hughes P, Van der Paal L, Whitworth J, Opio A, Grosskurth H. HIV prevalence and incidence are no longer falling in Southwestern Uganda: Evidence from a Rural Population Cohort 1989 – 2005. *AIDS* 2008; 22:1641-1649.

Paper 5. **Kamali A**, Nunn AJ, Mulder DW, Van Dyck, Dobbins JG, Whitworth JAG. Seroprevalence and incidence of genital ulcer infections in a rural Ugandan population. *Sexually Transmitted Infections* 1999; 75:98-102.

Paper 6. **Kamali A**, Kinsman J, Nalweyiso N, Mitchell K, Kanyesigye E, Kengeya-Kayondo JF, Carpenter LM, Nunn A, Whitworth JAG. A community randomized controlled trial to investigate impact of improved STD management and behavioural interventions on HIV incidence in rural Masaka, Uganda: trial design, methods and baseline findings. *Tropical Medicine and International Health* 2002; 7:1053-1063.

Paper 7. **Kamali A**, Quigley M, Nakiyingi J, Kinsman J, Kengeya-Kayondo J, Gopal R, Ojwiya A, Hughes P, Carpenter LM, Whitworth J. A community randomized trial of sexual behaviour and syndromic STI

management interventions on HIV-1 transmission in rural Uganda. *Lancet* 2003; 361:645-652.

Paper 8. Quigley AM, **Kamali A**, Kinsman J, Kamulegeya I, Nakiyingi-Miiro J, Kiwuwa S, Kengeya-Kayondo JF, Carpenter LM, Whitworth JAG. The impact of attending a behaviour intervention on HIV incidence in Masaka, Uganda. *AIDS* 2004; 18:2055-2063.

Paper 9. Ruzagira E, Wandiembe S, Bufumbo L, Levin J, Price MA, Grosskurth H, **Kamali A**. Willingness to participate in preventive HIV vaccine trials in a community-based cohort in south west Uganda. *Tropical Medicine and International Health* 2009; 14:196-203.

Paper 10. McCormack S, Ramjee G, **Kamali A**, Rees H, Crook AM, Gafos M, Jentsch U, Pool R, Chisembele M, Kapiga S, Mutemwa R, Vallely A, Palanee T, Sookrajh Y, Lacey CJ, Darbyshire J, Grosskurth H, Profy A, Nunn A, Hayes R, Weber J. PRO2000 vaginal gel for prevention of HIV infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. *Lancet* 2010; 376:1329-1337.

1.5. Thesis outline

The Introduction (chapter 1) describes the overall research programme, its development, a summary of the results from the published papers and the key role I played in the research. Chapter 2 critically examines the trends in HIV incidence and prevalence observed during the study period. Chapter 3 describes the interventions implemented to prevent HIV transmission in these cohorts and examines why some interventions have shown no impact on HIV transmission. Chapter 4 looks at gaps in our knowledge of HIV epidemiology in Uganda and suggests priority areas for future HIV research and interventions. Finally chapter 5 synthesises the answers to the research questions in the thesis based on my critical review of the research.

1.6. MRC/UVRI Uganda rural population based cohorts and studies

1.6.1. Study area and population

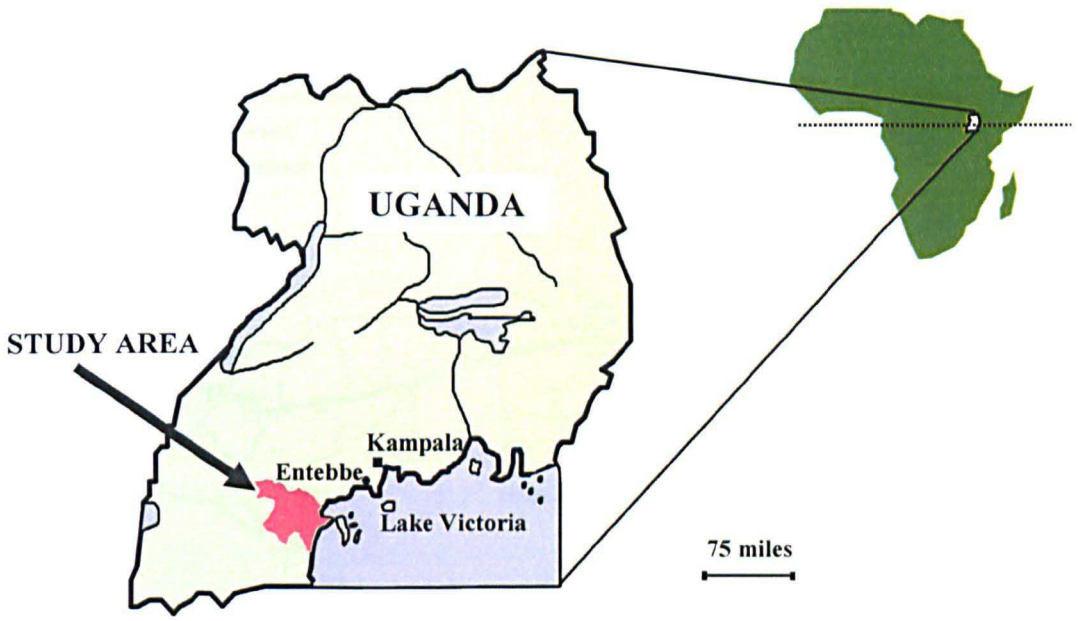
Uganda, a land-locked country located close to the equator in East Africa, is one of the countries that have experienced the most severe effects of the HIV epidemic. The country was initially regarded as the "epi-centre" of the HIV epidemic in Africa. HIV was first reported in 1983 on the shores of Lake Victoria in Rakai district spreading initially to the neighbouring district of Masaka and the rest of the country by 1986(15). It was partly because of this epidemiological background as well as local political support and the accessibility of the study area that Masaka district was chosen by the MRC/UVRI for the rural-based HIV epidemiological research and subsequent HIV intervention studies.

The study population where all the different studies were conducted comprised residents of Masaka rural district. The population was fairly stable with relatively low in- and out-migration. The district is located about 37 kms away from the Equator with an average altitude of 115m above sea level and the district capital (Masaka town) is about 120 km from Kampala. There are several ethnic groups, with the Baganda people predominating. The main language spoken is Luganda. The major economic activity in the district is agriculture with food crops (bananas, pineapples, and tomatoes), cash crops (coffee), cattle ranching, and fishing on Lake Victoria. There are industries that include coffee processing, soft drinks factories, and metal works.

The population of Masaka district is predominantly rural with a total of 831,300 people including 420,000 females and 411,300 males. They reside in scattered households with an average household size of approximately 6 persons. Of all residents approximately 50% are female and 56% are aged 13 years and below. Overall the population is predominantly Christian, with Roman Catholics and Protestants accounting for approximately 65% and 10% respectively. Only about 25% are Moslems. The proportions may vary slightly from one community to another.

Masaka district (Figure 1.1) was one of the first districts in Uganda to be hit by the HIV/AIDS epidemic and as a result many NGOs, both local and international, started work in the area from the late 1980's.

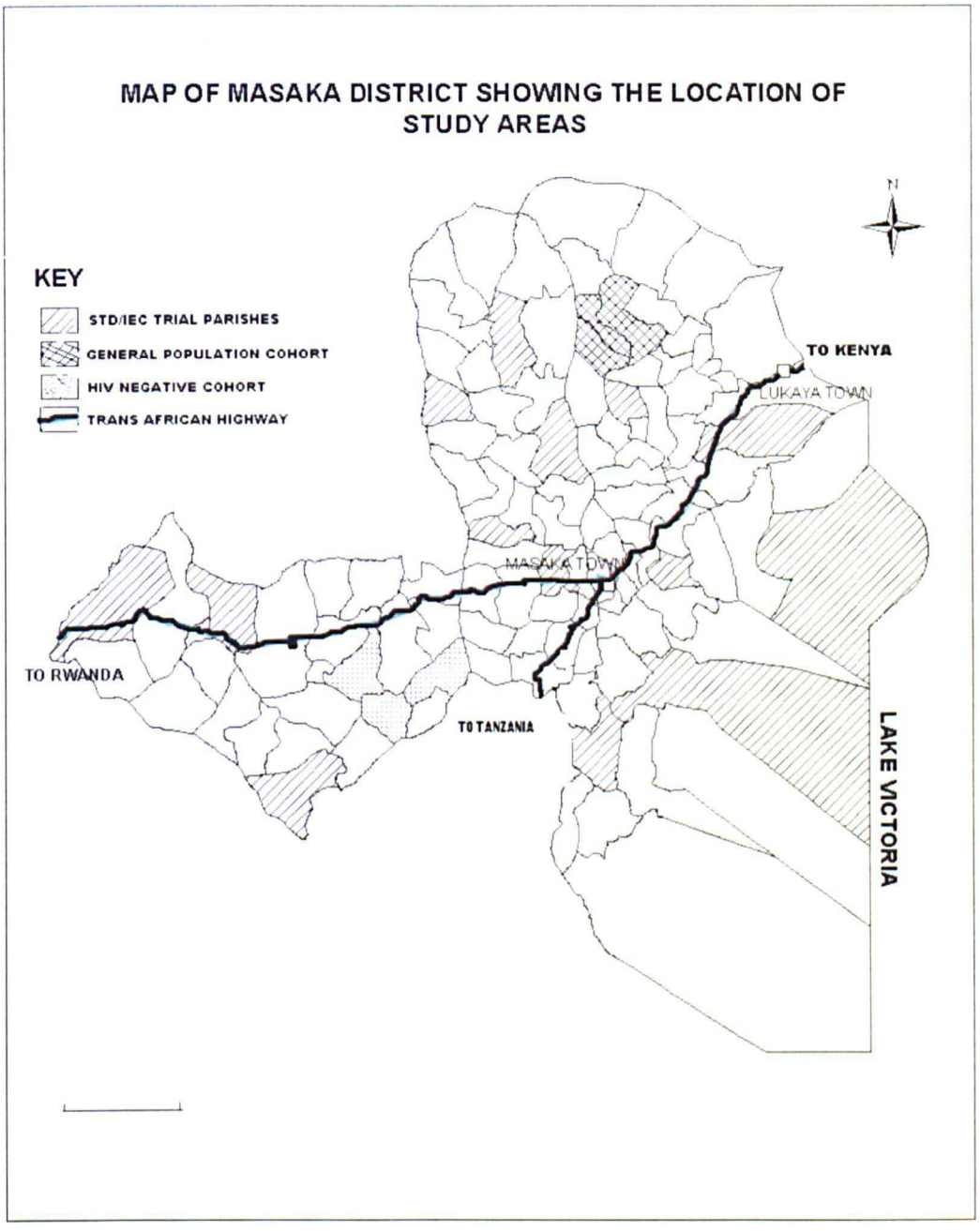
Figure 1.1 Map of Uganda showing Masaka district



1.6.2. Population cohorts and study designs

This thesis is based on data from a number of population based cohorts in Masaka district (Figure 1.2). I was instrumental in setting up and leading these cohorts (as described in section 1.8). Summarised in the following sections are the population cohorts in which the various studies were conducted. All cohorts were selected from rural populations in different parts of Masaka district and with no substantial differences in their characteristics.

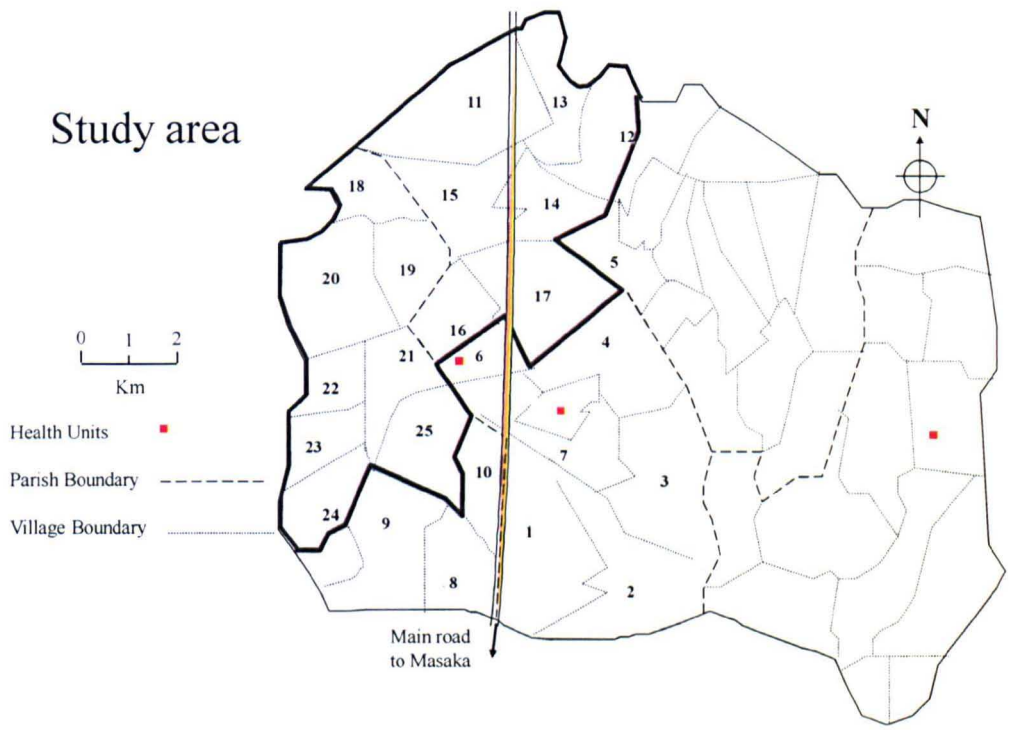
Figure 1.2 Map of Masaka district showing location study cohorts



1.6.3. General population cohort

The first cohort is a general population cohort, initially of approximately 10,000 people residing in 15 neighbouring villages established in 1989. The study area of the general population cohort is a rural sub-county situated about 32 km from Masaka town and 16 km from the international trans-African highway. At the 11th annual survey (1999) we aimed to increase accrual of HIV events in order to allow more reliable estimates of HIV incidence and prevalence and also to yield additional cases to be recruited into the former natural history cohort (now known as the rural clinical cohort). The cohort was thus expanded to cover an additional ten villages with a population of approximately 8,000. For logistical convenience, the new villages were adjacent to the old villages (figure 1.3) to the south and south east and in the same administrative unit (sub county). Seasonal dirt roads and footpaths connect all the villages. The characteristics of the new villages were generally similar to those of the old villages. One difference was that the new villages included the main trading centre of the sub county. A comparison of demographic characteristics between the sets of villages indicated that the residents from the new villages were more likely to have attained secondary or higher education, and to be separated or widowed, than those of old villages. Despite the fact that the old villages had been surveyed for 10 years and received HIV health education repeatedly, the HIV prevalence at the 11th annual survey was 6.1% overall, 5.7% in the new villages and 6.4% in the original villages (p-value=0.25)(16).

Figure 1.3 Map showing location of old and new study villages of the general population cohort



OLD Villages are numbered 11 to 25 (Enclosed in solid dark boundaries)
NEW Villages are numbered 1 to 10

Data from the general population cohort are included in papers 1-5 (12, 14, 17-19). Of these, only in paper 4 are data presented including both old and new villages. The overall aim of establishing this cohort was to document the course of the HIV epidemic and its biological and behavioural determinants in rural Uganda. The cohort has been under annual surveillance since late 1989. This includes annual mapping of the households in each village followed by collection of socio-demographic data (residence, migration and vital status of all residents)(20). A medical and serological survey follows the socio-demographic survey which includes administering an individual medical questionnaire, a brief physical examination and collection of a blood sample from all consenting individuals aged 13 years and above. HIV counselling and testing is offered to all those who provide a blood sample(9). The annual surveys are preceded by

community consultations and mobilization to explain the different aspects of the research and to seek community consent.

The cohort has provided valuable data for understanding the epidemiology of HIV in rural Uganda. These include estimates of baseline HIV prevalence by age and sex, and trends in annual incidence and prevalence, HIV-associated morbidity and mortality, HIV risk factors, and the socio-demographic impact of HIV. This knowledge subsequently led to setting up intervention cohorts in the district and designing studies to evaluate new strategies for the prevention of HIV infection.

1.6.4. STI/behavioural intervention cohort

The second cohort (STD intervention cohort), in the same district but geographically separated from the general population cohort described above, was established in 1994 to evaluate the impact of improved management of STDs on HIV transmission, with or without behavioural change interventions (Papers 6-8)(21-23) in 18 rural communities (approximately 96,000 adults). The trial aimed to determine the efficacy of Information, Education and Communication (IEC) alone and in combination with improved Sexually Transmitted Diseases (STD) management in reducing HIV transmission. Each community was a parish, a small administrative division of a district, with an average population of about 8,500 people. The study communities were about one hour apart by car which reduced the risk of contamination of interventions. This trial consisted of three arms with six communities in each arm. In arm A, a standardised behavioural intervention alone was implemented (community and school based). In arm B the same behavioural intervention as in arm A was implemented and in addition improved STD management was implemented through government and private health units. The STD component consisted of training health workers in all health facilities in syndromic management, counselling on risk reduction, partner notification, health education, regular supervision, and regular provision of essential supplies and drugs necessary for effective STD management.

The remaining 6 communities comprised the comparison arm C and received routine government health services, general community development activities related to income generating activities, and home-based care for elderly and bed ridden patients. In addition in arm C, health promotion seminars were conducted in each community on selected health topics (malaria, breast feeding, family planning and diarrhoeal diseases). The impact of the interventions was assessed through three Knowledge, Attitude, Behaviour, Practice (KABP) and serological surveys conducted at 18-24 months intervals in a sample of 750–1000 adults (13+ years) in each community. The sample was obtained by surveying 3–5 villages closest to the health unit.

1.6.5. High HIV prevalence cohort

Between 2002-2004, a third cohort of 1000 HIV negative adults from three rural high HIV prevalence communities (Figure 1.2) was established for conducting feasibility studies for HIV prevention trials including vaccine preparedness studies (Paper 9)(24). The study was conducted in three neighbouring rural communities in Masaka district, Uganda. The communities were selected to participate based on experience of HIV prevention research, previously high HIV prevalence, and presence of a health facility from which some study activities could be conducted. The HIV vaccine preparedness work comprised epidemiological studies to estimate baseline prevalence, incidence and predictors of HIV infection and studies to assess willingness to participate in preventive HIV vaccine trials (Paper 9)(24). First, a census of all residents in the three communities was conducted. Using the census lists, individuals were then contacted in person and invited to participate in the house-to-house survey. The survey was conducted one community at a time, until a sufficient sample size had been achieved. Individuals were enrolled if they were aged 18–60 years, HIV negative, clinically healthy and willing to give written informed consent, be tested for pregnancy (females), undergo sexual behaviour risk assessment, be counselled and tested for HIV and receive test results.

At enrolment, study information was given, written informed consent obtained and an interviewer administered questionnaire used to collect information on demographics, vaccine knowledge, sexual risk behaviours and medical history. Blood was drawn for

HIV serology. Female participants were asked to provide a urine sample for pregnancy testing.

HIV incidence in this cohort was shown to be similar to that in the general population cohort and the STD intervention cohort (approximately 1-2%) and was thus considered not high enough for designing future intervention trials whose end point is often HIV incidence.

1.6.6. HIV high risk (discordant couple) cohort

A high risk cohort of approximately 1300 HIV discordant couples was established, part of which was used for recruiting HIV negative female partners in a large scale phase III microbicide trial (Paper 10)(25). The discordant couples were identified through house-to-house VCT surveys within Masaka town as well as clinics offering VCT services within Masaka district. The rationale for this new focus on higher HIV risk cohorts was to prepare for future HIV biomedical intervention trials that would aim to reduce the risk of HIV transmission. Recruitment of HIV discordant couples was therefore considered a possible solution to avoiding the large sample size that would be a consequence of using cohorts with a low HIV incidence rate.

The purpose of these high risk cohorts was therefore to:

- prepare the ground for intervention studies by collecting population based background information required to conduct large-scale clinical trials
- assess whether participants would accept the intervention and study procedures prior to starting efficacy trials
- conduct studies to evaluate the effectiveness of innovative intervention strategies (microbicides and HIV vaccines), among HIV negative adults living in HIV discordant couples

The couples were recruited through house-to-house surveys that identified individuals in stable heterosexual relationships. Consenting individuals were offered couple HIV counselling and testing. Test results were only provided after couple counselling. The

HIV positive partners were provided with necessary and appropriate investigations (including CD4 count), as well as treatment and referral to an HIV care support organization of their choice for initiation of anti-retroviral treatment, if required.

1.6.7. Vaginal microbicides

Between 2002 and 2005 a feasibility study to assess the suitability of a discordant couples' cohort for a phase III efficacy trial of vaginal microbicides was conducted(26). The study recruited approximately 120 discordant couples identified from VCT clinics around Masaka town, and followed every 3 months for one year. This provided us with an opportunity to: (i) estimate HIV incidence; (ii) evaluate trial procedures such as the provision of informed consent, genital examination, acceptability of and adherence to the study product using a placebo gel and (iii) monitor sexual behaviour using case record forms, coital diaries and in-depth interviews.

On completion of the microbicide feasibility study in 2005, a phase III international multi-centre, randomised, double-blind, placebo-controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO 2000/5 gels for the prevention of vaginally acquired HIV infection was started (Paper 10). The trial, code-named MDP 301, screened participants between September 2005 and August 2008 and enrolled 9385 out of 15,818 screened at 13 clinics, which were managed by six research centres in four African countries (South Africa, Tanzania, Uganda and Zambia)(25). Sexually active HIV-uninfected women aged 18 years or older (≥16 years in Tanzania and Uganda) were randomly assigned in a 1:1:1 ratio to 2% PRO2000, 0·5% PRO2000, or hydroxyethylcellulose placebo gel groups for 52 weeks follow up (up to 104 weeks in Uganda). The study was conducted by the Microbicides Development Programme (www.mrc.ac.uk/mdp), which is a collaboration of African and European institutions. The MDP301 Uganda clinical site enrolled 840 HIV negative healthy women in a known HIV sero-discordant couple relationship. These were part of the HIV discordant couple cohort. The women were followed up to at least 52 weeks and to a maximum of 104 weeks. Women were enrolled between September 2005 and August 2008. Follow-up continued up to September 2009. Women were included if they were sexually active,

aged ≥16 years, without HIV-1 infection, were willing to be tested for HIV-1 infection and receive the result, to have regular speculum examinations and urinary pregnancy tests, to use the allocated gel as instructed, and to receive health education about condoms and able and willing to give informed consent. They were ineligible if they were unable or unwilling to provide a reliable method of contact, were likely to move out of the area within 12 months, were likely to have sex more than 14 times a week on a regular basis (a regulatory requirement was that no more than 60 applicators were to be dispensed at every 4 weekly visit), used spermicides regularly, were pregnant or within 6 weeks post-partum, had a severe clinical or laboratory abnormality; needed referral for assessment of a suspicious cervical lesion, had received treatment to the cervix or other gynaecological procedure within 30 days of enrolment, were allergic to latex; or were participating or had participated in another clinical trial that was likely to affect the primary efficacy endpoint within 30 days before enrolment.

1.7. Laboratory and statistical methods

All sera from consenting participants both in the general population and the intervention (high risk) cohorts were tested for HIV antibodies using two independent enzyme linked immunoassays to establish HIV status (Wellcozyme HIV-recombinant, Murex Biotech, Dartford, UK; and Recombigen HIV-1/2, Trinity Biotech, Galway, Ireland) with set algorithms and with confirmation by Western blot, (Cambridge Biotech Corporation, Rockville, USA)(27). We tested for sexually transmitted infections (STIs) in the various studies using the most recent standard assays at the time of the study.

To estimate HIV infection rates in the general population cohort, age- and sex-specific prevalence was derived from the numbers of adults testing HIV positive and negative at each survey round. Incidence rates were calculated for all adults with an HIV negative result who provided a repeat blood sample on one or more occasions in subsequent surveys. Person-years at risk for HIV incidence commenced at the date of the initial HIV negative test and ceased at the date of sero-conversion. The date of seroconversion was calculated as the midpoint between the last negative and first positive HIV result. In

examining trends in HIV infection rates, analyses were conducted at three time points, after seven, ten and fifteen years of cohort follow up.

Analyses for the STD/behavioural intervention trial were done separately for each of the intervention arms (A and B) and compared to the control arm C. The HIV incident events and person-years at risk of seroconversion were combined over all survey rounds. Analysis of STI prevalence and reported sexual behaviour at round 2 or round 3 was restricted to persons who had provided data for at least two survey rounds. All results were adjusted for potential confounders (age, sex, baseline prevalence for outcome measures, religion and history of genital ulcers), and analyses carried out using appropriate statistical methods for cluster randomized trials (CRT).

In the MDP 301 trial, HIV status was confirmed using serum obtained up to 6 weeks before enrolment, at enrolment, and then at weeks 4, 12, 24, 40, and 52 (and weeks 72 and 104 in Uganda). The HIV testing algorithm involved confirmation of all HIV positive results at a central laboratory in South Africa. The central laboratory analysed samples from the visit at which a positive serological rapid test result was obtained and from all previous visits at which samples were obtained. This approach was due to the limitations of serological assays in which a woman may already have been infected at enrolment but not yet positive on serological tests. The trial was designed to have 80% power to detect a 35% reduction in HIV incidence, assuming an incidence of 4.0 per 100 woman-years in the placebo group. Women were censored at the date of seroconversion, estimated by the midpoint between the last negative test and date of detection, or at the last HIV-negative test for patients who did not become infected. The primary efficacy endpoint was analysed as time-to-event, and intervention groups were compared by use of hazard ratios (HRs) relative to the placebo group. Safety endpoints were analysed as time-to-first-event and groups compared as for the efficacy analysis.

1.8. My contribution to this research

I was involved in setting up the rural general population cohort from the start of the study, and was responsible for the field activities from 1989-1993 and thereafter was overall team leader up to 1997. My specific responsibilities were to formulate research topics for each annual survey, design the necessary research tools such as questionnaires, supervise and ensure high quality data collection, and contribute to data management, analysis and publication of research findings.

I took over the leadership and direction of the STD/Behavioural intervention trial half way through the study in 1997 up to 2001 when the study was completed. My tasks were to ensure adequate delivery of the interventions to the communities, achieve high follow up of the participants during the second and third survey rounds, and timely completion of the trial. I took the lead in ensuring data quality, initiated analyses and published baseline findings, process evaluation of the interventions and the final results. At the end of the intervention trial, I was involved in a collaborative project to explore the reasons for the contrasting results of the three East African STI trials(22, 28-29).

The idea of setting up high risk cohorts of HIV discordant couples was entirely my own. I was the principal investigator for the HIV vaccine preparedness studies and the microbicide feasibility studies, and responsible for both the scientific direction and day-to-day management of these studies. I was also the Ugandan clinical site principal investigator for the microbicide studies.

1.9. Summary of findings from the publications on which this thesis is based

1.9.1. HIV infection rates and trends

The general population cohort provided the first data on HIV infection rates in rural Uganda and subsequently an opportunity to monitor epidemiological trends, in the

absence of any biomedical interventions other than health education and provision of STI treatment services in government health clinics using national STI syndromic management guidelines and discussed in Papers 1-4(12, 14, 18-19). As already mentioned papers 1-3 are based on data from the original 15 villages and only paper 4 includes data from both new and old study villages.

HIV prevalence at baseline (1989/90) among adults (13 years and above) was 8.2% with the highest rates occurring in women aged 20-24 years (20.9%) and in men aged 25-34 years (18.1%)(12). ***Paper 1*** describes seven-year trends in HIV infection rates, and changes in sexual behaviour(12). In paper 1, the analysis of age- and sex-specific prevalence was based on the number of adults testing HIV positive and negative at each survey round, in contrast to analyses reported in paper 2 which used imputation methods. HIV prevalence declined from 8.2% at baseline (1989-1990) to 6.9% in 1996-1997 ($p=0.008$) with the greatest decline observed among men aged 20-24 years (11.7 to 3.6%; $p<0.001$) and in women aged 13-19 years (4.4% to 1.4%; $p=0.003$) and 20-24 years (20.9% to 13.8%; $p=0.003$). Prevalence however increased among women aged 25-34 years (13.1 to 16.6%; $p=0.04$). The overall incidence declined from 7.7/1000 person years [pyrs] (95% CI 4.9-12.2) to 4.6/1000 (95% CI 2.8-7.6) during this period, but neither this nor the changes in age-sex specific incidence rates were significant(12).

In ***paper 2*** trends in HIV incidence and prevalence are examined using data over 10 years (up to 1998/1999) from the same cohort(18). In this paper HIV prevalence was calculated using all definitive HIV serological results for each round. Individuals censused but not bled at a particular round were classified as HIV negative if they tested seronegative at a later round and positive if they had tested positive at an earlier round. This method of imputation enabled us to assess HIV status for some individuals who were censused but not bled at particular rounds. This method, however, underestimates the prevalence at the earliest rounds and overestimates the prevalence at later rounds. In particular the baseline prevalence was estimated as 8.2% (derived from data at first survey round) and 6.9% (using the imputation method), and the latter is likely to be an underestimate.

A significant decline in overall HIV prevalence was observed (p=0.03) especially among young women; from 2.8% to 0.9% in those aged 13-19 years and from 19.3% to 10.1% in those aged 20-24 years. In men, significant declines were seen in those aged 20-24 years (6.5% to 2.2%) and 25-29 years (15.2% to 10.9%). However there was a significant rise in HIV prevalence among women aged 30-34 years (10.7% to 20.6%) and 35-39 years (8.3% to 14.7%); no rise in prevalence among men was observed. Overall HIV incidence for all adults declined from 8.0 in 1990 to 5.2 per 1000 pyrs in 1999 (p=0.002), with an average annual reduction of about 0.45 per 1000 pyrs. A significant decline in incidence was observed in both females and males of all ages(18-19). This was the first evidence of a significant decline in HIV incidence in a rural general adult population in Africa.

In ***paper 3***, HIV prevalence and incidence trends were calculated over the same 10 year period as in paper 2, but using cross-sectional measures for each survey round without extrapolating data from previous surveys (for HIV positive individuals) or subsequent surveys (for HIV negative individuals)(19). The observed incidence rates were similar to those reported in paper 2 (above) and overall prevalence as well as trends were also comparable to those measured using the imputation method.

In ***paper 4***, we analysed HIV trends over 15 years(14). The encouraging trends in HIV infection observed at earlier survey rounds were no longer evident when using data over 15 years of follow up(14). The decline in HIV prevalence was only observed up to 1999/2000, from 8.5% in the 1990/1991 survey round (a year after baseline survey) to 6.2% in 1999/2000. Thereafter the rate rose to 7.7% in 2004/2005. Overall HIV prevalence fell during the 1990s but started increasing from 1999/2000 (p=0.01 for significance of change in trend). Similarly, incidence fell through the 1990s, but between 1998 and 2004 incidence rates were no longer falling but instead there were indications that they were rising.

Subjects in longitudinal cohort studies such as the MRC cohort may not participate at

each time point of follow up for several reasons such as absence at the time of survey or research fatigue. This could potentially lead to inaccurate and biased estimates of trends over time. One approach to overcome this is to impute the missing data so to generate a more complete data set. Data on HIV status were imputed such that all individuals who were HIV positive at any previous survey round were classified as such at subsequent survey(s), if still present, irrespective of whether they were HIV tested or not. Similarly for individuals who were present at a given round and HIV negative, their HIV status was imputed as HIV negative for previous rounds if they were present but not tested.

This approach has the advantage that it uses all available data during the entire follow up and provides more reliable estimates. One possible disadvantage of this method is that it may underestimate the prevalence of HIV-1 at the earliest rounds and overestimate the prevalence at the later rounds since it only imputes HIV negative data (denominator) backwards and HIV positive data (numerator) forwards. Results from data analyses using imputation may differ from those using repeated cross sectional data analyses if the compliance rates at individual survey rounds vary greatly and also if there is a difference in participation between HIV negative and positive individuals. We however noted in Papers 2 and 3 that the prevalence trends measured by both approaches gave similar results.

1.9.2. HIV risk factors

The general population cohort provided us with an opportunity to assess risk factors for HIV infection in rural Africa during the early years of the epidemic. ***Paper 5*** (based on data from the ten old villages) describes the sero-prevalence and incidence rates of *Treponema pallidum, Haemophilus ducreyi* and *Herpes simplex* Virus type-2 (HSV-2), and the association between HIV infection and the incidence of these STIs(17). The sero-prevalence of *T pallidum* was 12.9% among males and 12.6% among females. The corresponding rates for *H ducreyi* were 9.8% and 7.3% respectively. The prevalence for HSV-2 was considerably higher, 36.0% in males and 71.5% in females. We also observed a strongly significant association between HIV prevalence and sero-prevalence of *H ducreyi* (p=0.01), and HSV-2 (p=0.004) but not with *T pallidum(17).* This

observation was consistent with other epidemiological and biological evidence suggesting that STIs enhance the transmission of HIV(30-31). Incidence rates for *T pallidum* were 8.4 per 1000 pyrs for males and 12.3 for females; the rates for *H ducreyi* were 24.6 and 20.0 and for HSV -2 were 73.2 and 122.9 per 1000 pyrs respectively. The rate ratio (RR) of HSV-2 incidence was 6.69 (95% CI 2.06-6.61) in initially HIV seropositive individuals versus HIV seronegative after adjusting for age and sex. The RR for *H ducreyi* was 3.50 (95% CI 1.29-9.46) among HIV positive females versus negatives but there was no effect in males. There were too few incident cases for *T pallidum* to make meaningful comparison. A number of other risk factors have been documented in this rural cohort and briefly these were: age, 13-21 years in women (Odds Ratio [OR] 8.6 (95% CI 3.0-24.5); marriage in those aged <25 years, OR 2.3 (1.5-3.7); working outside one's village among those aged ≥25 years, OR 1.8 (1.2-2.6). Moslems were at lower risk than non-moslems (those aged 13-24 years, OR 0.58 (0.35-0.95) and ≥25 years, OR 0.58 (1.2-2.6))(32).

1.9.3. HIV prevention efforts

Globally, there have been attempts to control HIV transmission and a range of biomedical HIV intervention strategies have been developed and evaluated through randomized controlled trials (RCTs) including our own STD/behavioural intervention trial. These include treatment of bacterial STIs, behavioural interventions, male circumcision, treatment of HSV-2, vaginal microbicides, Pre Exposure Prophylaxis (PrEP) and HIV vaccines. To date seven RCTs have shown efficacy against HIV transmission, of which 3 were on male circumcision which showed approximately 60% protection for heterosexual males(33-35). The RR (95% CI) were 0.49 (0.30-0.82) in the Rakai trial, 0.47 (0.28-0.78) in the Kisumu trial, and 0.40 (0.24-0.68) in the South Africa trial. One RCT evaluated syndromic STD case management at primary health care level and showed a significant reduction in HIV incidence of 42% (RR 0.58 [95% CI 0.42-0.79])(28). Another was the Thailand HIV vaccine trial that showed modest benefit with an efficacy of 31.2% (95% CI, 1.1%-52.1%)(36). Two recent trials were the CAPRISA 004 trial that reported a 39% (95% CI, 6%-61%) reduction using 1% tenofovir vaginal microbicide gel(37) and the iPrex trial using oral Truvada among men who have sex

with men (MSM) that demonstrated a 44% (95% CI, 15%-63%) reduction in HIV incidence(38).

The HPTN052 trial looking at ARV treatment as prevention among ART naïve patients with a CD4+ cell count of 350-550 cells/mm^3 in a number of countries has recently reported a 96% reduction in the risk of HIV transmission to the uninfected partner among HIV serodiscordant couples (www.hptn.org).

In ***paper 6*** we describe the trial design, methods and baseline findings of the STD/behavioural intervention trial(21). In summary, the study design was a three-arm community randomized trial of 18 rural communities. The intervention was targeted at the resident population (96,000 adults). Impact assessment of the interventions was through three questionnaire and serological surveys conducted at 18-24 months intervals. The required survey sample size for impact evaluation in each arm was 4500 adults (750-1000 adults in each community). Obtaining this number required our surveying 3-5 villages closest to a health unit. It was necessary to survey whole villages and not part of a village. Out of approximately 20,000 resident adults in the survey villages, 14,500 (72%) were enrolled for the impact assessment. Of those enrolled 97% were both tested for HIV and interviewed at baseline. The primary outcome measure was HIV incidence and secondary measures were syphilis and *Herpes simplex* virus type-2 (HSV-2) incidence, prevalence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, and reported sexual behaviour. HIV baseline prevalence ranged between 8.8% in arm A and 10.4% in arm C. The demographic and behavioural characteristics were also similar. There was little variation between arms in STD prevalence for active syphilis (range 10.8%-14.8%), HSV-2 (range 27.9%-28.4%), gonorrhoea (range 1.1%-1.4%) and Chlamydia (1.5%-2.0%)(21).

Paper 7 discusses the main results of the STD/behavioural intervention trial. In summary the HIV incidence did not differ between the behavioural intervention (group A) and comparison groups (group C) or between the STD intervention (group B) and comparison groups even after adjusting for various covariates(22). Compared to the

comparison arm the incidence rate ratios (in brackets 95% CI) of HIV were 0.94 (0.60-1.45) in group A and 1.00 (0.63-1.58) in group B, and the corresponding prevalence ratios for condom use with last casual partner were 1.12 (0.991.25) and 1.27 (1.02- 1.56) respectively(22).

In ***paper 8***, we did further analyses of these results which indicated that although the behavioural intervention had no significant benefit in the communities as a whole, it resulted in a reduced risk of HIV acquisition among women who attended at least one intervention activity compared to those who attended none (adjusted rate ratio 0.41 [0.19-0.89])(23).

At the end of the trial comparative analyses were conducted using data from this trial and those from two other similar trials in the region, the Mwanza syndromic STI and Rakai STI mass treatment intervention studies(39-40).

1.9.4. Preparations for future HIV prevention efficacy trials

As HIV transmission continues especially in low-income countries, the development of new prevention methods such as HIV vaccines and microbicides is a high priority for research even in the era of ART rollout. In preparation for these efficacy trials, feasibility studies were initiated to assess the suitability of population cohorts. It was necessary to assess whether such cohorts would provide sufficiently high HIV incidence rates for inclusion in efficacy trials, and to examine the retention and willingness to participate in these cohorts. In ***Paper 9*** we assessed willingness to participate in HIV vaccine efficacy trials as well as motivations, concerns, and barriers to participation among volunteers completing two years of follow up in a HIV vaccine preparedness study in the general population, in communities with high HIV prevalence(24). A high level of willingness to participate in such trials was observed. The key motivating factors were: hope of being protected from HIV (47%), access to regular HIV counselling and testing (36%) and altruism (28%). However a number of trial requirements (delayed pregnancy among women, giving large volumes of blood) were associated with reduced willingness to participate(24).

1.9.5. Vaginal microbicide prevention trial

Paper 10 discusses the results of the Microbicide Development Programme (MDP) phase III trial of the 0.5% and 2% PRO 2000/5 vaginal microbicide gels(25). This was a multi-centre trial in 4 African countries, with Masaka district in Uganda as one of the sites. Participants were instructed to apply a single dose of study gel up to one hour before every act of vaginal intercourse using a single-use, pre-filled applicator (the study gel contained either the microbicide or a placebo, depending on the arm of the trial). Participants received free condoms and risk-reduction counselling, regular health checks, sexually transmitted disease testing and treatment, and referral for HIV treatment where appropriate. The primary outcome measures were HIV infection and grade 3 (severe) or 4 (life-threatening) adverse events. The secondary outcomes included HSV-2, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections.

Overall gel adherence in the trial was high (mean reported gel use at last sex act was 89%) and did not differ between the three gel arms. Reported condom use at last sex act increased over time from 57% at enrolment to 88% at week 52 of follow up, and was similar across gel groups though there was variation between research centres. HIV incidence (primary outcome) was similar between groups at the end of the study- 4.5 per 100 woman years (95% CI 3.8-5.4) in the 0.5% gel arm and 4.3 (3.6-5.2) in the placebo arm, HR 1.05 (0.82-1.34), p=0.71. The Data Safety Monitoring Committee of the trial had earlier recommended discontinuation of the 2% gel arm due to futility. At the time of the 2% arm closure, HIV incidence was 4.7 (3.8-5.8) in the 2% gel arm, 3.9 (3.0-4.9) in the 0.5% gel arm and 3.9 (3.1-5.0) in the placebo arm. The primary safety endpoint was a grade 3 (severe) or worse clinical or laboratory adverse event. The rates of safety endpoints did not differ between study arms either at the end of the study or at the time 2% gel was discontinued. Both PRO 2000 gel concentrations were safe but not effective against vaginal HIV transmission.

In the following chapters I shall critically examine epidemiological trends of HIV infection in Uganda, the key determinants of these trends and strategies that could prevent HIV in this population. To this end, I shall introduce some new material that

was not included in the original, published papers.

1.10 Key determinants of HIV incidence and prevalence

There are a number of key determinants for HIV sexual transmission that are broadly categorised into socio-economic and cultural (income, education, occupation, religion and ethnicity), demographic (sex, age, marital status, mortality and migration), behavioural and biological. The socio-economic, cultural and demographic factors can often be viewed as underlying determinants of HIV transmission whereas the behavioural and biological factors are those that directly determine the rate of new infections (exposure of susceptible to infected person, transmission probability per sexual contact and duration of infectiousness).

Because of the complexities of these multiple factors, and their inter-relations, a proximate-determinants framework used in demographic studies (fertility and child survival), has been suggested to help in understanding the drivers of HIV transmission and how they relate to each other (41). Briefly, the framework suggests that the ***underlying*** determinants (eg age, sex, marital status, etc) influence the risk of transmission through ***proximate*** (ie behavioural and biological) determinants such as the presence of other sexually transmitted diseases, sexual mixing and concurrent partnerships, condom use, antiretroviral treatment, and lack of circumcision among men. Without such linkage the underlying factors would not on their own affect HIV transmission. Based on the published papers included in this thesis, the following underlying and proximate determinants have been examined to understand HIV trends in this cohort:

Underlying determinants	*Proximate determinants*
Age	Number of sexual partners
Sex	Age at first sex
Marital status	Condom use
Migration	Antiretroviral treatment
Mortality	

Previous analyses of data from this cohort have also looked at other determinants such as socio-economic factors, lifetime sexual partners, and religion. Briefly there was evidence of increased risk of HIV infection with poverty among heads of households, greater number of sexual partners (more than four), and not being a Muslim suggesting a possible protective effect of circumcision(42-43).

It has not been possible to examine other determinants due to the nature of the study design that assessed HIV infection through annual house-to-house surveys. These include determinants that facilitate sexual exposure to HIV (frequency of sex acts, sexual mixing with different population patterns); and those that enhance transmission (sexual practices e.g anal sex, circumcision status, presence of STIs and their treatment). This would have required additional data collection, for example on presence of sexually transmitted infections and infectiousness of infected partners (viral load) or more in-depth social science techniques, that were not feasible in the context of this research. Contributions of other determinants (blood transfusion, injection drug use, unsafe injections) have also not been examined because of epidemiological evidence that these modes of transmission do not play a major role in HIV epidemic in this setting(44-45).

Other limitations with the approach used here are related to the well known reporting biases associated with sexual behaviour data collection. In addition some of the data were not systematically collected at every survey and not on all sexually active individuals. For example, selected sensitive questions on sexual behaviour were incorporated in some annual survey rounds, but were not repeated in all rounds due to

fear of research fatigue that could report in greater reporting bias. Retrospective application of this framework to existing data is another limitation in that data on some important parameters were not collected or are difficult to assess particularly the underlying determinants such as social, economic and environmental factors that are linked to HIV transmission through their effects on proximate behavioural and biological determinants.

Chapter 2. Trends in HIV incidence and prevalence

Summary

This chapter examines trends in HIV prevalence and incidence in Uganda and considers possible explanations for these trends. Overall HIV prevalence was higher for females than males in all years in the MRC cohort. The prevalence declined in both men and women between 1990 and 2000 but rose between 2000 and 2008. The decrease between 1990 and 2000 was particularly pronounced for those aged 20-24 years. However, for those aged 35 years and above, a steady increase in HIV prevalence was observed among females and males throughout the study period (1990-2008). Various reasons for these age and sex differences in HIV prevalence are explored in the chapter.

Trends in HIV incidence rates in the MRC cohort were less clear than the trends in HIV prevalence. Overall HIV incidence (all ages) declined significantly between 1990 and 2008 ($p=0.001$). The decline in incidence was significant between 1990 and 2000 ($p=0.01$) although there was a non-significant increase from 2001 to 2008 ($p=0.34$). A decline in HIV incidence was seen from the early 1990s, indicating that the decline could have started earlier, possibly in the late 1980s.

I used a proximate determinants framework to better understand trends in HIV prevalence. A net outflow of HIV positive migrants, more deaths of HIV positive individuals and reductions in risky sexual behaviour in the 1990s seem to have been important factors in explaining the decline in HIV prevalence during that decade. The increase in prevalence since 2000 is not fully understood. Improved survival due to ART, the "normalization" of HIV/AIDS and possible complacency around safer sex could have led to an increase in risky sexual behaviour.

2.1. Introduction

This chapter examines trends in HIV prevalence and incidence rates in Uganda during the study period (1989-2008), considers possible determinants of these trends and compares them with trends in two other countries in East and Southern Africa.

For this chapter I have conducted additional analyses by extending the analysis to 2008. I have applied imputation of data from all survey rounds by extrapolating data from previous surveys for HIV positive individuals and from subsequent surveys for HIV negative individuals. As a consequence there are minor discrepancies between the estimates presented in this chapter and the point estimates in chapter 1 which are based on the published papers and analyses conducted at the time the papers were written. In this chapter, I have used four age groups (13-19, 20-24, 25-34 and ≥35) for describing the age and sex-specific HIV prevalence trends. Due to relatively small numbers incidence trends have not been analysed by age group.

Uganda was one of the earliest countries in Africa to report the new epidemic of HIV when Serwadda and colleagues first reported the spread of "Slim Disease" along the shores of Lake Victoria in 1982(15). The epidemic that started silently spread rapidly across the country mainly along the trans-African and other major highways. Due to civil unrest at the time there were no prevention efforts until 1987 when the first HIV prevention programme was started through the National AIDS Control Programme. However this was at a time when the country was already facing a major epidemic. The first published HIV prevalence data in Uganda indicated a prevalence rate in the early 1990s of 30-40% in urban areas(46) and around 8-10% in rural districts(32, 47).

As summarized in chapter one, data from the MRC cohort study on which this thesis is based indicated that HIV prevalence rates fell during the 1990s(19). However this decline was no longer evident from 2001(14). In this chapter, I describe in more detail trends in HIV prevalence and incidence among those individuals residing in the old

villages that participated in annual surveys of the MRC cohort from 1990 up to 2008 as well as examining data from other sources. Specifically, I examine:

- trends in HIV prevalence by age and sex in the MRC cohort as well as HIV prevalence at a national level;
- trends in HIV incidence by sex in the MRC cohort and HIV incidence estimates from other sources in Uganda;
- trends in sexual behaviour in the MRC cohort as well as in other studies;
- possible explanations for the time trends in HIV prevalence;
- possible explanations for the age-sex differences in HIV prevalence
- HIV prevalence trends in two other African countries.

2.2. HIV prevalence

2.2.1. Time trends in HIV prevalence in the MRC cohort

Little was known about HIV/AIDS in Uganda and in many other parts of sub Saharan Africa prior to the mid-1980s. This was because of low awareness, fear of being tested for HIV and stigma. Most people with HIV were only reported near the time of death. Data on HIV/AIDS initially came only from urban hospitals in large cities. National surveillance systems were non-existent and there were no community based studies on HIV. Consequently no reliable data on time trends in HIV prevalence were available before 1990.

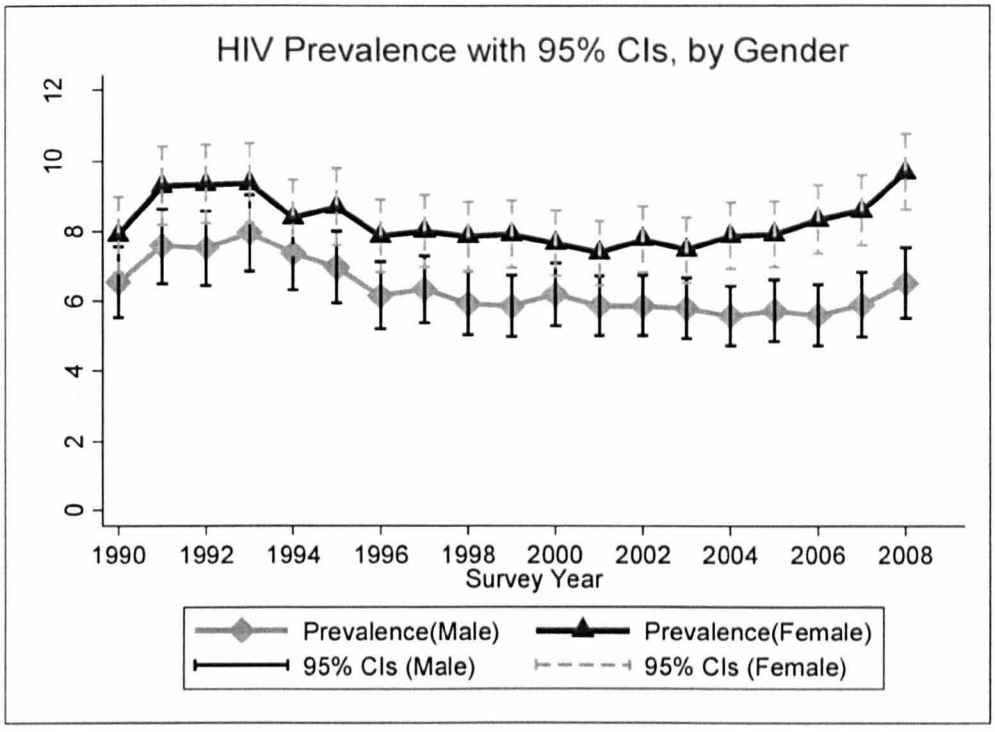
Using data from the rural population cohort of the MRC Programme, we found that overall HIV prevalence declined in both men and women between 1990 and 2000, from 6.5% to 6.2% (p value for trend <0.01) and from 7.9% to 7.7% (p value for trend <0.01), respectively (table 2.1 and figure 2.1). Trend analysis for HIV prevalence over the entire study period (1990-2008) indicates a significant decline among men (p for trend < 0.001) but a non significant decline among women (p for trend = 0.29). In all years HIV prevalence was, overall, higher for females than males.

An initial rise in prevalence was observed up to 1993 followed by a decrease thereafter. The decline in HIV prevalence that started in 1993/4 continued up to 2000/1, an encouraging observation that contributed to the Uganda "success story" through the 1990s. Between 2001 and 2008, prevalence increased from 5.9% to 6.5% (p value for trend =0.5) in males and from 7.4% to 9.7% (p value for trend <0.01) in females. Overall HIV prevalence remained higher for females than males in all years.

Table 2.1 HIV prevalence by gender (1990-2008) with 95% CI

	Males				Females			
Survey	**HIV+**	**N**	**Prev (%)**	**95% CI**	**HIV+**	**N**	**Prev (%)**	**95% CI**
1990	149	2278	6.54	5.53-7.56	191	2416	7.91	6.83- 8.98
1991	177	2335	7.58	6.51-8.65	239	2568	9.31	8.18-10.43
1992	178	2366	7.52	6.46-8.59	239	2555	9.35	8.23-10.48
1993	189	2375	7.96	6.87-9.05	238	2536	9.38	8.25-10.52
1994	174	2361	7.37	6.32-8.42	216	2571	8.4	7.33- 9.47
1995	165	2364	6.98	5.95-8.01	221	2538	8.71	7.61- 9.80
1996	146	2370	6.16	5.19-7.13	202	2566	7.87	6.83- 8.91
1997	155	2446	6.34	5.37-7.30	213	2657	8.02	6.98- 9.05
1998	153	2578	5.93	5.02-6.85	220	2796	7.87	6.87- 8.87
1999	155	2645	5.86	4.97-6.76	232	2928	7.92	6.95- 8.90
2000	170	2744	6.2	5.29-7.10	236	3078	7.67	6.73- 8.61
2001	164	2799	5.86	4.99-6.73	230	3108	7.4	6.48- 8.32
2002	159	2707	5.87	4.99-6.76	238	3058	7.78	6.83- 8.73
2003	158	2725	5.8	4.92-6.68	230	3076	7.48	6.55- 8.41
2004	150	2690	5.58	4.71-6.44	242	3070	7.88	6.93- 8.84
2005	147	2565	5.73	4.83-6.63	240	3027	7.93	6.97- 8.89
2006	146	2473	5.6	4.71-6.50	251	3007	8.35	7.36- 9.34
2007	146	2473	5.9	4.97-6.83	257	2984	8.61	7.61- 9.62
2008	152	2328	6.53	5.53-7.53	283	2918	9.7	8.62-10.77

Figure 2.1 HIV prevalence by gender (1990-2008)



2.2.2. Age and sex differences in HIV prevalence in the MRC cohort

Figures 2.2 and 2.3 show trends in HIV prevalence by age and sex. Prevalence among young men aged 13-19 years remained generally low and stable (below 1.0%) throughout the study period. Among men aged 20-24 years there was a significant decline from 8.7% in 1990 to 1.9% in 2000 (p value for trend <0.001) and thereafter a non-significant rise to 2.3% in 2008 (p value for trend=0.47). A decline was also observed among those aged 25-34 years, from 15.9% in 1990 to 14.2% in 2000 (p value for trend =0.002) followed by further decline to 10.4% in 2000 though non-significant. In men aged 35 years and above however, there was a borderline significant increase in prevalence from 6.7% in 1990 to 9.7% in 2000 (p value=0.05) and thereafter a non-significant rise from 9.1% to 11.5% in 2008 (p value for trend = 0.12), reaching the same level as in those aged 25-34 years.

Figure 2.2 HIV prevalence by age among males (1990-2008)

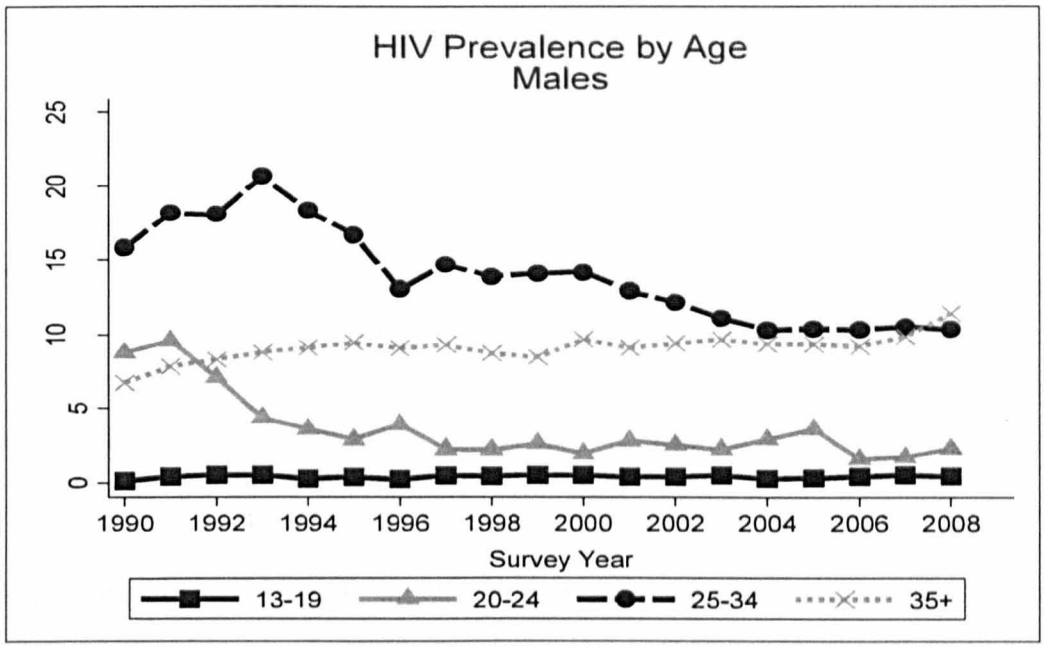
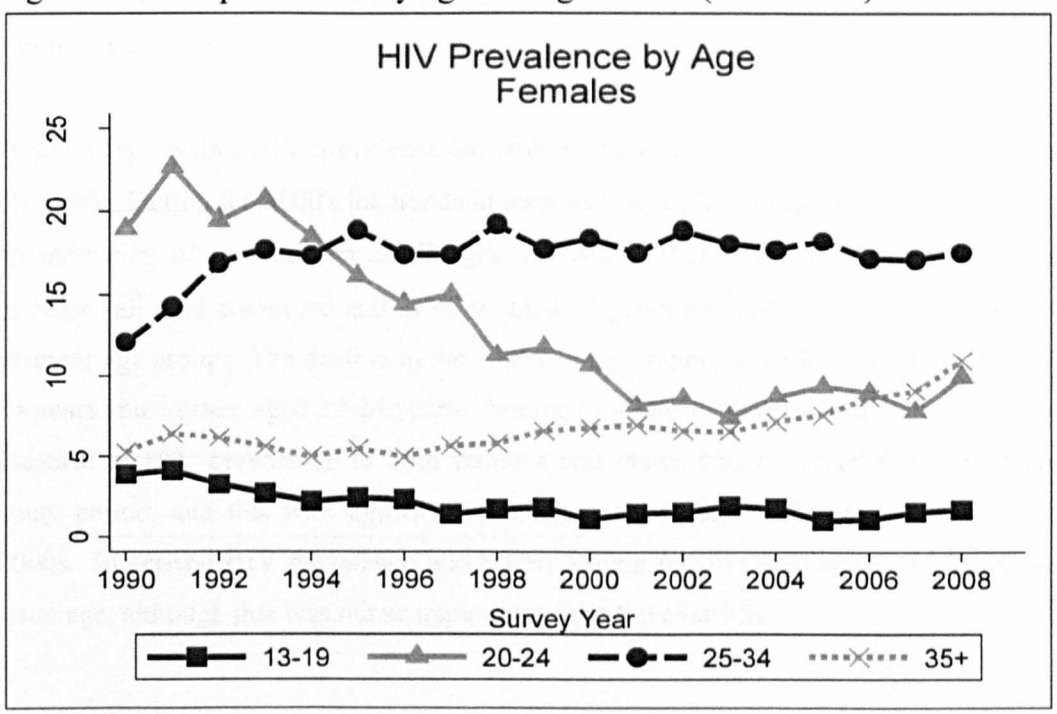


Figure 2.3 HIV prevalence by age among females (1990-2008)



Prevalence in women aged 13-19 years declined from 3.9% to 1.1% (p value for trend <0.001) during the 1990s and then increased up to 1.7% in 2008 (p value for trend < 0.001). Despite very high prevalence among women aged 20-24 years at baseline (figure 2.3) there was an encouraging decline from 19.0% in 1990 to 10.7% in 2000 (p value for trend <0.001) with a significant increase in the 2000s (from 8.1% in 2001 to 10.0% in 2008). In women aged 25-34 years, however, there was an increase in prevalence during the 1990s from 12.1% at baseline to 18.4% in 2000 which was significant (p value for trend =0.005). Thereafter the rates remained fairly stable in this age group through the 2000s. The trends among women aged 35 years and above have probably been the most discouraging with no decline but rather a steady and significant rise during both periods (p value for trend < 0.001). This was clearly the opposite of what was seen among those aged 20-24 years. The rates among women aged 35 years and above reached the same level as those aged 20-24 years by 2006 (figure 2.3).

In all years of follow up, HIV prevalence among females aged 13-19 and 20-24 years exceeded that of males. A female excess was also seen among those aged 25-34 from 1996 onwards. Among those aged 35 years and above, prevalence was similar for males and females

In summary, overall HIV prevalence for both men and women declined significantly in the 1990s. During the 2000's the trends in men were less clear though there seemed to be an indication of stabilization at all ages. In women there was an overall significant increase (all ages combined and in those aged 35 years and above) but no increase for younger age groups. The decline in the 1990s was particularly marked for men aged 20-34 years and women aged 20-24 years. Among those aged 35 years and above, a steady increase in HIV prevalence in both females and males was observed throughout the study period, and this was significant among males in the 1990s and women in the 2000s. In general HIV prevalence was higher among females than among males of the same age, although this was not so apparent among the over 35s.

2.2.3. HIV prevalence at a national level

At a national level the analysis of HIV prevalence trends revealed three distinct phases; initially a phase of rapid increase between 1989 and 1992 with prevalence peaking at about 18%, followed by a rapid decline between 1992 and 2002 and finally a phase of stabilization at about 6.1-6.5% between 2002 and 2005(48). The trends in HIV prevalence observed within our cohort have been seen in other studies in Uganda such as the Rakai Programme cohort and the antenatal surveillance monitoring system of the Ministry of Health. HIV prevalence in the Rakai cohort showed a significant decline between 1994/1995 and 2002/2003 in those aged 15-49 years, from 19.7% to 12.9% (a 34% reduction) among women and from 15.0% to 9.3% (a 38% reduction) among men(49). In the antenatal clinics, HIV prevalence trends in Northern Uganda showed a significant decrease from 26.0% in 1993 to 16.1% in 1997 ($p < 0.001$). The decrease was more pronounced among women below 30 years ($p < 0.001$) in both rural and urban areas(50). From the surveillance system established in Uganda in 1989, data from six urban antenatal sentinel sites (two in Kampala and four from other major towns) indicated that overall prevalence in all clinics peaked at 15-30% in 1991-92, followed by a decline to 5-12% by 2002; this decline was seen mainly among women aged 15-19 and 20-24 years. In Kampala-based clinics alone, antenatal HIV prevalence declined from 30% in 1992 to 8.3% in 2002(51). Data from the 2004-2005 National Sero-Behavioural Survey indicated overall prevalence of 6.4% and 6.3% among those aged 15-49 and 15-59 years respectively. These studies reflect the overall downward trend in HIV prevalence reported by the MRC cohort between 1990 and 2000, although the evidence for a subsequent increase during the 2000s is less clear.

2.3. HIV incidence

2.3.1. Time trends in HIV incidence in the MRC cohort

Data on HIV incidence rates are more useful in tracking the epidemic, as well as evaluating interventions, than HIV prevalence since incidence rates give an indication of the current course of the epidemic. The trends in incidence rates observed from 1990-

2008 in the MRC cohort are less clear than prevalence trends mainly because the incidence estimates are based on smaller numbers of HIV seroconversions than the numbers of HIV infections used to estimate prevalence. Because of small numbers, two-year incidence estimates have been computed to monitor the trends in the MRC cohort (table 2.2 and figure 2.4). Even using this approach, the confidence intervals around these estimates are very wide. Overall the incidence (all ages) decreased from 7.5 in 1990 to 6.4 per 1000 pyrs in 2008 (p=0.001). The decline in incidence was significant between 1990 and 2000; from 7.5 to 4.6 per 1000 pyrs (p=0.01). However there was a non-significant increase from 2001 to 2008 (5.8 to 6.4 per 1000 pyrs, p=0.34). In men incidence rates declined from 9.2 per 1000 person years (pyrs) in 1990 to 5.4 per 1000 pyrs in 2000 (p=0.17), and from 4.6 in 2001 to 3.5 per 1000 pyrs in 2008 (p=0.31). The rates among women declined from 5.9 in 1990 to 4.0 per 1000 pyrs (p=0.04) in 2000. The corresponding rates between 2001 and 2008 were 6.8 to 6.5 (p=0.62) respectively. The decline in incidence seen from the early 1990s could be an indication that the decline could have started earlier, possibly in the late 1980s. This decline continued through the early 2000s.

Earlier analyses of annual incidence rates from this cohort had indicated that HIV incidence declined steadily for all adults from 8.0 per 1000 pyrs in 1990 to 5.2 per 1000 pyrs in 1999, p=0.002(14, 18-19). The declining trend was observed in both females and males of all ages as well as in specific age groups (13-34 years and those aged above 35 years).

Figure 2.4 HIV incidence rate by gender 1990-2008

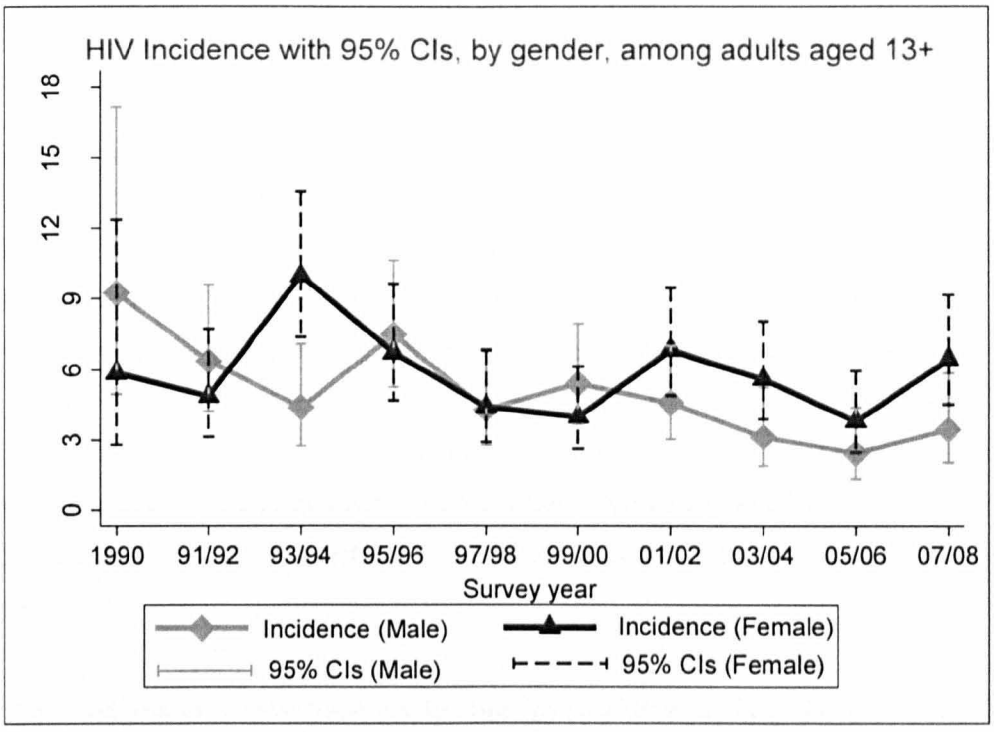


Table 2.2 HIV incidence rate by gender 1990-2008 with 95% CI

	Males				Females			
Period	**Cases**	**PYO†**	**Inc/1000**	**95% CI**	**Cases**	**PYO†**	**Inc/1000**	**95% CI**
1990	10	1082.6	9.23	4.97-17.17	7	1187.6	5.89	2.81-12.36
1991/92	23	3613.3	6.37	4.23-9.58	19	3867.6	4.91	3.13 -7.70
1993/94	17	38.56.7	4.41	2.74-7.09	41	4106.3	9.99	7.35-13.56
1995/96	31	4151.7	7.47	5.25-10.62	30	4457.0	6.73	4.71 -9.62
1997/98	19	4397.8	4.32	2.76-6.77	21	4715.0	4.45	2.90 -6.83
1999/00	26	4804.6	5.41	3.68-7.95	21	5230.0	4.02	2.62 -6.16
2001/02	22	4778.9	4.6	3.03-6.99	36	5265.5	6.84	4.93 -9.48
2003/04	15	4762.8	3.15	1.90-5.22	30	5336.6	5.62	3.93 -8.04
2005/06	11	4517.6	2.44	1.35-4.40	20	5195.8	3.85	2.48 -5.97
2007/08	14	4028.5	3.46	2.06-5.87	31	4798.0	6.46	4.54 -9.19

† Person-years (PYO)

Whereas HIV prevalence rates among males were significantly lower than in females throughout the study period, we did not see any sex differences in incidence. This discrepancy is rather surprising, as it would be expected that women would have higher incidence due to behavioural and biological factors that predispose women to greater HIV susceptibility (see 2.6.1). One possible explanation could be that when we began to monitor trends in HIV incidence in this cohort (late 1989), incidence rates had already started declining. Women may have had a higher incidence than men until the early 1990s which could explain their higher prevalence subsequently. Thereafter incidence rates are likely to have been too low in both sexes to be able to detect any male-female differences. On average the number of incident cases in the MRC cohort was approximately 22 cases annually in both sexes. With such small numbers it would be difficult to detect any sex difference in incidence even when data over time periods are combined.

The trend analyses have been carried out for two time periods, the 1990s and 2000s. This approach was taken for a number of reasons. We had already used similar cut off points in some of the previous published papers in which we reported a decline from 1990 to 2000 and thereafter a rise from 2000 to 2005(14, 19). Similar periods have also been used in other studies that have described trends in HIV prevalence in Uganda(52). Secondly it appeared logical to analyse the trends during the first 10 years of the study and then compare with later years. The first ten years was a period when there were intensive national HIV prevention campaigns which, together with effects resulting from deaths of relatives, friends and in wider communities, could have resulted in reductions in risk behaviour. On the other hand, there were reports of complacency for HIV prevention at individual and organizational levels in the later years during the 2000s. Antiretroviral treatment became available in mid 2000s and may also have contributed to complacency.

I acknowledge that the periods chosen for these trend analyses could be considered as an arbitrary choice of cut off dates and possible alternative periods could be used. There are

also other alternative approaches that future analyses could consider, such as change-point analysis. This is considered to be an effective and powerful statistical tool when analysing historical data and determining if and when a change in a data set has occurred. This method characterises the changes, controls the overall error rate and provides a confidence level that indicates the likelihood of the change(53).

2.3.2. HIV incidence data from other sources

Data on trends in HIV incidence in Uganda have only been available from two longitudinal cohorts - the MRC cohort and the Rakai cohort. The first evidence of falling HIV incidence in Uganda was provided by data from the MRC cohort over the 10-year period 1990-2000(14, 18-19). Data from the Rakai cohort however showed that there was no significant decline in HIV incidence in men and women aged 15-49 years or in young adults aged 15-24 years between 1994/1995 and 2002/2003(49). The reasons for this discrepancy between these two studies, carried out in neighbouring districts, are not clear though it is possible that the epidemic is older in Rakai than in Masaka district. Rakai district which borders Tanzania is where the first AIDS cases were identified and has been thought to be the initial epicentre of the HIV/AIDS epidemic in Uganda. It is therefore possible that the peak of HIV incidence in Rakai was some years before Masaka and by the 1990s the decline had levelled off.

In summary, trends in HIV incidence rates are less clear than those for HIV prevalence. For all adults we observed a significant decline in HIV incidence rates in the 1990s and a non-significant increase from 2001. The declines seen in men during the two time periods were not significant. In women there was a borderline significant decline in the 1990s and a non significant increase in the 2000s. These observations could be related to the stage of the HIV epidemic in Uganda and clearer patterns could possibly have been found earlier in the epidemic before surveillance of this cohort began. It is not yet clear if incidence is now increasing, because of the small annual number of HIV seroconversions.

2.4. Trends in sexual behaviour

2.4.1. Data from the MRC cohort

It is important to examine whether trends in HIV infection may be explained by trends in behaviour in the same population. I will focus mainly on specific behavioural factors that have been demonstrated to be associated with HIV risk in previously published work based on data from the same rural MRC general population cohort. We observed that HIV prevalence was associated with reported number of lifetime sexual partners but not with number of partners in the past 12 months. HIV prevalence increased with number of lifetime sexual partners, with an OR of 2.1 among those with 4-10 partners and 4.9 among those reporting ≥ 11 partners(42), compared with those reporting 1-3 partners ($p < 0.001$). Similarly, we observed that the strongest risk factor for HIV incidence in this same cohort was lifetime sexual partners. A significantly higher risk of HIV infection was observed in both men (OR 3.8) and women (OR 20.8) with ≥5 lifetime sexual partners compared to those who reported at most one partner(54).

HIV transmission in sub-Saharan Africa is predominantly heterosexual and changes in sexual behaviour contribute to epidemiological trends in HIV infection. Collection of sexual behaviour data started in survey round 4 (1992/3). We avoided asking sensitive sexual behaviour questions in early survey rounds as there was not sufficient research capacity but also to initially build community confidence in the research team. In addition, we did not ask all sexual behaviour questions at every survey. This was to reduce fatigue among study participants and hopefully to avoid misreporting on sensitive questions, if asked annually. For example, age at first sex was asked in 1992/3 (round 4) and thereafter annually. Data on condom use at last sex were first collected in 1992/3, then in 1996/7, 1997/8, and annually from 1999 to 2001, and then in 2003, 2005 and 2006.

Initial analysis of sexual behaviour data from the cohort indicated that risky sexual behaviour declined in the late 1990s(12). A comparison of sexual behaviour at survey rounds 4 and 8, when age at first sex was asked, indicated that the median age at first

sex among young men aged 13-19 years was 17.5 years in 1992/3 compared to 18.2 years in 1996/7 (p value for trend =0.002) (figure 2.5). However median age at first sex among young women aged 13-19 years did not change over that time (16.7 years in both survey rounds, p value for trend=0.2). There was also a significant decrease in the annual age-specific prevalence of being married for young women aged 16-19 years (p value for trend <0.001). This was particularly so between 18 and 19 years. There was no change in age at first marriage for men. The median age at both survey rounds was 24 years for men (figure 2.6).

Figure 2.5 Percentages who reported ever having sex in survey rounds 4 and 8 ages 13-19

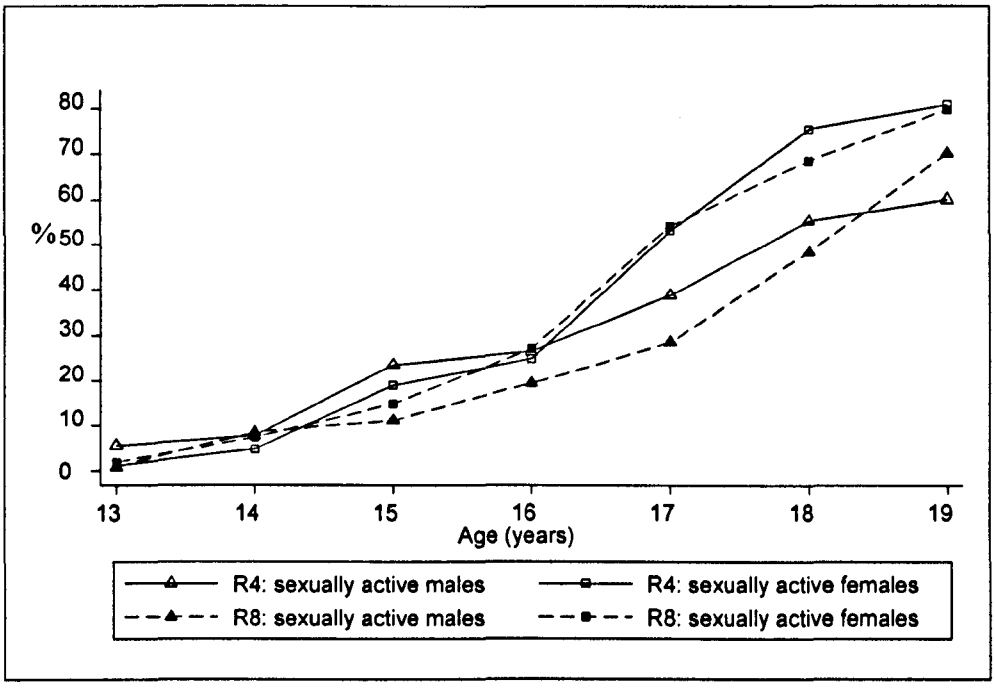
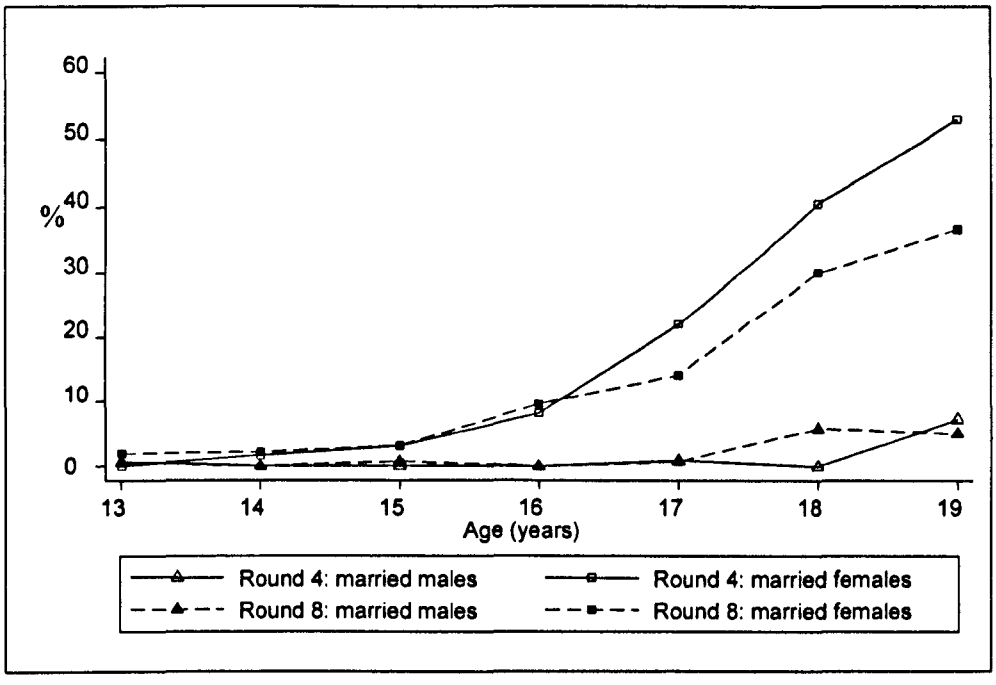


Figure 2.6 Percentages who were married in survey rounds 4 and 8 ages 13-19



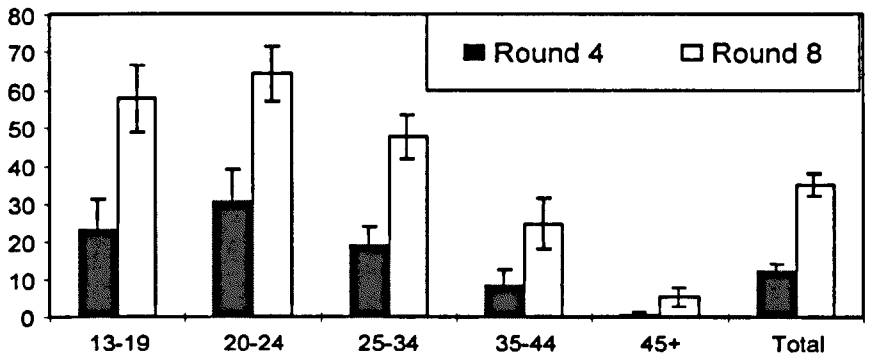
Another paper from this cohort described sexual behaviour between 1993 and 2006(55). Briefly there were trends towards safer sexual behaviour in the late 1990s and early 2000s among teenagers and young adults. Among those aged 17–20 years, reported median age at first sex increased by almost a year from 17.8 years to 18.6 years between 1997 and 2006 (p for trend, 0.001). Among girls it increased from 16.7 to 18.2 years and among boys from 18.5 to 19.9 years. The proportion of 13–19 year olds who reportedly had never had sex increased from 71.7% in 1993 to 82.6% in 2006. In addition there was an increase in secondary abstinence (defined as being sexually active at least once, but to have not been sexually active in the past year) in the 13-19 year olds in both boys and girls but no change in other age groups. Similarly there was an increase in age at first marriage among both women and men aged 13-29 years from 19.0 to 19.4 years and from 24.9 to 25.3 years respectively. In general, these trends in sexual behaviour among teenagers and young adults reflected HIV prevalence trends in the same period for this age group.

The rate of condom use also increased significantly between 1992/3 and 1996/7 (figure 2.7). The overall proportion who had ever used condoms increased in males from 12.5% to 35% during the five year period (p value for trend <0.001) and in females from 4% to 12% (p value for trend <0.001), with significant increases for all age groups under 35 years (figure 2.7). The observed trends towards increase in ever using a condom continued up 2006: in men from 35% in 1996/7 to 46.1% in 2006. The corresponding rates among women were 12% and 30.7% respectively.

Figure 2.7 Reported use of ever using condoms by sex and age at survey rounds 4 (1992/3) and 8 (1996/7) with 95% CI (restricted to those ever having had sex)

a) Males

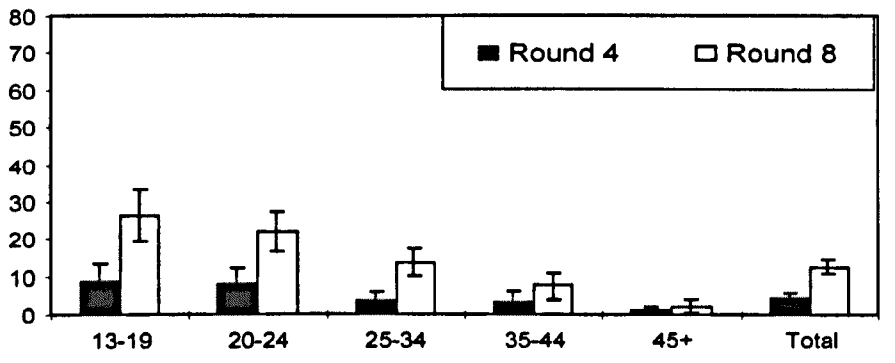
%



Age group

b) Females

%



Age group

Condom use at last sex act with any partner decreased in men from 18.9% in 1996 to 15.3% in 2000 and 14.7% in 2003, and then increased to 15.6% in 2005 and 18.1% in 2006 (p for trend <0.001). Among women the rates were 5.7% in 1996 increasing to 8.6% in 2000, then a decrease to 8.0% in 2003 and 7.6% in 2005, followed by an increase to 9.9% in 2006 (p for trend =0.71). Among married people who reported any casual partner in past year, condom use at last sex with casual partner was 67% in 1997 (when question was first asked), 50% in 2000, 55% in 2003 and 59% in 2005 (data not

available in 2006), p for trend =0.02. The rates in men were 71% in 1997, 59% in 2000, 61% in 2003 and 63% in 2005. Married women who reported a casual sex partner were less likely to use a condom at last sex (40% in 1997, 8% in 2000, 24% in 2003 and 38% in 2005). The sex-specific trends in condom use at last sex with a casual partner among married people were not significant. There was an overall drop in proportion of married people reporting condom use at last sex with a casual partner in 2000 especially among women. It is possible that these were underestimates. Trends of sexual behaviour using data on numbers of sexual partners from the cohort were not consistent during the study period to make any meaningful interpretation.

The research team provided HIV testing and counselling to the residents in the study area as well as continuous health education. This was in addition to the National AIDS control programme activities. Any decline in risky behaviour in our cohort could therefore have been a result of the national control efforts of HIV-specific health education from governmental and non-governmental sources and in the media (private local radio stations and newspapers) as well as MRC research activities.

2.4.2. National data

Data on sexual behaviour from the Uganda national Demographic Health Survey (DHS) conducted in 1988/89, 1995 and 2000/01 indicate that the median age at first sex among women increased from 16.5 years in 1989 to 17.3 years in 2000, and among men from 17.6 years in 1995 to 18.3 years in 2000. In addition the proportion of those reporting premarital sex and multiple sex partners among women decreased between 1995 and 2000 though there were no changes in reported extramarital sex among men(51). Comparison of 2000/01 and 2004/5 DHS indicated that the median age at first sex among those aged 20-24 increased among women from 16.7 to 17.1 years, and declined among men, from 18.8 to 18.3 years.

Trends in sexual behaviour observed in the DHS (1989, 1995 and 2000) suggested a shift towards less risky behaviour(56). The percentage of people who reported ever using a condom gradually increased in both women and men from 1989 to 2000. Among

women (aged 15-49 years) it increased from 1% to 16% and in men (aged 15-54 years) from 16% to 40%.

2.4.3. Summary of trends in sexual behaviour

In summary, there was a trend towards less risky sexual behaviour between 1990 and 2000 in the MRC cohort which continued up to 2006 particularly in young adults. There was an increase in median age at first sex in males, in the proportion of those who reportedly had never had sex and in secondary abstinence in both males and females. We also observed an increase in age at first marriage in both sexes aged 13-29 years. The trends in condom use are less clear. The findings from the Uganda DHS between 1989 and 2005 also indicated trends towards less risky behaviour.

2.5. Explaining time trends in HIV prevalence in Uganda

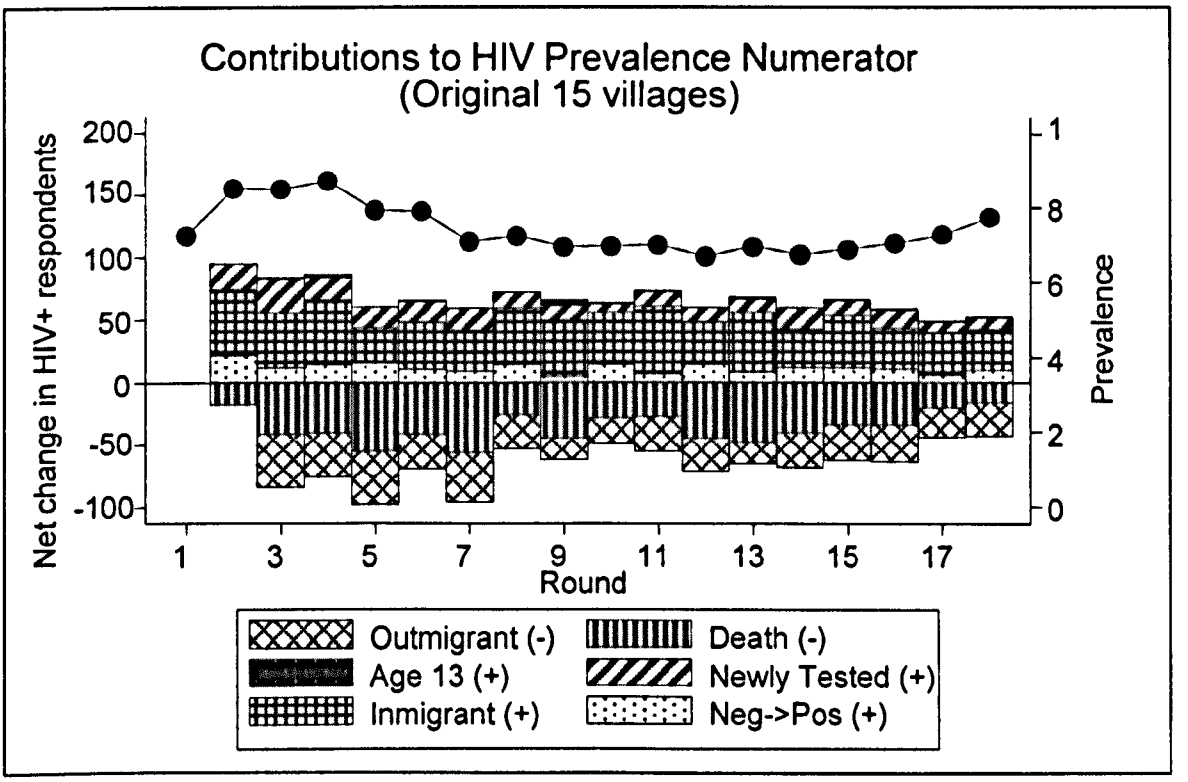
2.5.1. Factors that can influence HIV prevalence

Understanding how HIV prevalence changes from one survey to the next in the MRC cohort is complex, because it depends on a number of factors. HIV prevalence could increase for the following reasons: an increase in the number of people sero-converting from the previous round (increase in incidence); an increase in survival due to improvements in treatment; an increase in the number of HIV-positive people moving into the area (in-migration); an increase in the number of people who have never previously tested testing positive (reducing the undiagnosed fraction). On the other hand, HIV prevalence could decrease from one round to another because of the death of prevalent cases, because of HIV-positive people leaving the area (out-migration) or because of a decrease in HIV incidence. I will explore underlying determinants such as migration and mortality as well as proximate determinants such as reported sexual behaviour in trying to explain the trends in HIV prevalence observed during the study period.

2.5.2. Increasing HIV prevalence 1990-1993

The increase in HIV prevalence during the initial survey rounds (1990-1993) was largely due to the in-migration of people with HIV infection as well as people newly testing for HIV (figure 2.8).

Figure 2.8 Contributions to HIV prevalence numerator



In 1990, health services in rural Uganda were generally inadequate whereas the MRC Programme was offering free treatment to study participants. Consequently, seriously ill patients, many of whom could have been HIV infected, returned to their natal home from urban and neighbouring areas to access treatment that was being offered at no cost to study participants.

In addition the MRC research group was new to the area in 1990 and the population was initially suspicious, leading to selective participation in the study. The sick, some of whom could have been HIV positive, were less likely to participate at first because of their fear that serological surveys were aimed at identifying HIV infected individuals and injecting them with a lethal chemical. Secondly, the amount of blood drawn was thought to be substantial and many sick individuals declined to consent to bleeding in the initial survey. However as the MRC survey established strong community mobilization and networks, and employed local staff from the study villages these fears diminished and more people participated, many of whom had never had a previous HIV test.

Thus, despite the apparent increase in HIV prevalence from 1989 to 1993, it is possible that when HIV surveillance started in 1989 in this population prevalence of HIV had already started declining.

2.5.3. Declining HIV prevalence up to 2000

What caused the decline in HIV prevalence in Uganda from the early 1990's to 2000? There are several factors that could have contributed to this decline. In many epidemics, it is possible for disease incidence to peak because the most susceptible people get infected first and then to fall because of the depletion of susceptible subjects (the natural course of the epidemic). This is followed by a fall in prevalence. In Uganda, estimation of HIV incidence through modelling has indicated a peak in incidence in the mid-1980s, followed by a rapid reduction in the later 1980s but no further substantial reduction through the 1990's(57). In the MRC cohort, the number of new HIV infections over the 10-year period 1989-1999 was approximately 190, with an overall average annual reduction in incidence of about 0.45 per 1000 person years of observation. Though small, this reduction in incidence could have made some contribution to the decline in prevalence among males up to 2000.

Migration and mortality are some of the underlying determinants in the proximate-determinants framework that could explain these trends. As shown in figure 2.8, the

decline in prevalence for all ages particularly in the mid-1990s was a result of a net outflow of HIV positive migrants and more deaths of HIV positive individuals. The greatest decline in prevalence was observed in young people aged 20-24 years, though this cannot be explained by mortality alone. HIV infected individuals in this age group would have recently acquired HIV infection with a low mortality rate during the study period. Out-migration would have to be higher in HIV positive than HIV negative individuals to reduce HIV prevalence in this age group though this is plausible since "mobile" individuals are more likely to acquire HIV. There seems to have been very little change in incident cases during the years when prevalence decline was most evident.

Changes in sexual behaviour patterns (abstinence, age at first sex, number of sexual partners and condom use) are proximate determinants that could also offer possible explanations for the decline in HIV prevalence. It has been suggested that Uganda's success story was a result of the reduction in sexual risk behaviour, the evidence for which I have presented in this chapter. For example, we observed an increase in both primary and secondary abstinence among teenage boys and girls. Between 1992 and 1997, the median age at first sex among teenagers increased ($p=0.002$) among males, but there was no change in females. The age-specific prevalence of being married decreased among females aged 16-19 years but there was no change in males. Ever use of condoms (for all ages) increased significantly between 1992 and 1997 among males but there was no change among females. We also observed a decrease in condom use at last sex with a casual partner among married people, though we did not observe clear trends in the number of sexual partners.

These reductions in risky sexual behaviour were a consequence of political support and openness about HIV in the late 1980s, the establishment in 1987 of the first ever national AIDS Control Programme (ACP) followed in 1992 by the multi-sectoral Uganda AIDS Commission in 1992, and the strong HIV prevention campaigns delivered by these organisations. The ACP introduced new policies, expanded partnerships, promoted public health education for behaviour change, strengthened sexually

transmitted disease management, improved blood transfusion services, as well as care and support services for persons with HIV/AIDS(58). The ACP model of community mobilization and communication enabled HIV campaigns to reach the general population and target groups of all ages in both rural and urban areas. Equally important was the recognition and respect of those already infected with HIV. This was mainly through the creation of various indigenous care and support organizations for HIV positive individuals particularly The AIDS Support Organisation. These organizations and key individuals have played an important role in advocating against stigma and discrimination. The establishment of voluntary counselling and testing (VCT) centres such as the AIDS Information Centre together with openness around HIV provided encouragement for the general public to access VCT. The impact this alone could have played in reducing HIV prevalence is hard to estimate. However, combined with other interventions such as improved diagnosis of, and treatment of sexually transmitted infections, it must have contributed to the observed decline. Unlike other African countries this high level of political support and muti-sectoral approach appears to have been special to Uganda.

2.5.4. Increasing HIV prevalence from 2000

Why was the declining trend in HIV prevalence up to 2000 not maintained? There are several explanations that could be considered; underlying and as well as proximate determinants will be considered.

Firstly, were there major changes in the population structure such as the migration of HIV positive and high risk people into the MRC study area at about the time prevalence began to increase? Similar epidemiological trends have been observed across the country, supported by data from national sentinel surveillance and these observations were not unique to this cohort alone(11). Moreover, during the 2000s, there were no changes in the proportions of HIV positives among in and out migrants to the study area (figure 2.8), and overall no net in-flow of migrants. The main mode of transmission has remained predominantly heterosexual and there have been no reported changes in HIV

transmission patterns in sub Saharan Africa(59) to explain the decline in HIV prevalence.

Were there methodological changes over time in the MRC study? The data collection methods have remained unchanged over time with the same field survey team, some of whom have been following the cohort since the first survey in 1989. Similar laboratory testing algorithms using two different EIA systems (Recombigen HIV-1 EIA and Wellcozyme HIV-1 Recombinant) have been maintained throughout the study period. Similarly the survey coverage rate has been approximately 70% annually. Therefore there have been no differences in data quality to suggest any methodological biases that could explain the changes in HIV trends.

Could the HIV molecular epidemiology in this cohort have changed in recent years to explain the reversal in the epidemiological trends? Data on HIV subtypes in this population reveals that subtypes A and D are the most prevalent with a few recombinant viruses. No significant changes in the subtypes of HIV infection in the cohort have been observed in this cohort(60).

What has been the effect of introducing antiretroviral therapy (ART) in this cohort? There is evidence that ART reduces viral load both in blood and genital secretions(61-62). This potentially decreases infectiousness and incidence(63) but also leads to a reduction in mortality which would increase prevalence over time. ART was introduced in the MRC cohort to those with CD4 < 250 cells/mm^3 in 2004, according to the Ministry of Health ART guidelines, and by the end of 2008 about 200 patients had started treatment. Consequently the introduction of ART could potentially have contributed to the rise in prevalence from the mid 2000s because of improved survival. Indeed there was an observed reduction in number of deaths in the later years of the 2000s (figure 2.8).

Patterns of sexual behaviour from 2000 onwards among older adults (25-44 years) are inconsistent(55) and cannot fully explain the increase in HIV prevalence. For example,

condom use at last sex act with any partner decreased in men from 18.9% in 1996 to 15.3% in 2000 and 14.7% in 2003, and then increased to 15.6% in 2005 and 18.1% in 2006 (p for trend <0.001). Among women the rates were 5.7% in 1996 increasing to 8.6% in 2000, then a decrease to 8.0% in 2003 and 7.6% in 2005, followed by an increase to 9.9% in 2006 (p for trend 0.71).

Though the sexual risk behaviour changes from mid 2000 have not been well demonstrated there is evidence that people's perceptions regarding HIV/AIDS and sexual behaviour are changing for various reasons. Risky sexual behaviour among HIV-infected people has been shown to rise in this cohort with some indications that ART availability may also increase risky sexual behaviour among HIV-uninfected individuals(64). It is possible that the initial fear of AIDS related to the deaths of close relatives and friends during the early years of the epidemic may have declined after more than two decades. On the other hand, the inconsistent trends in sexual behaviour from 2000 onwards suggest that the relationship between the availability of ART, changing attitudes towards HIV and sexual behaviour is not straightforward.

There has also been more emphasis within various HIV/AIDS programmes towards treatment and a shift from the strong HIV prevention strategies of the early 1990s together with less funding for HIV prevention. It is believed that the strong political support for the ABC strategy in the early years of the epidemic changed in the early to mid 2000s. There was a shift within the ABC strategy towards US-based abstinence-only initiatives and less comprehensive sex education and condom promotion. These factors could partially explain the observed rise in prevalence trends, although trends in sexual behaviour after 2000 are less clear than those before 2000.

HIV transmission in stable relationships is another factor that could have driven the epidemic among older people in whom rate of condom use is very low. The HIV discordance rate among married couples in Uganda is estimated to be about 5-7%(65). The 2004-2005 Uganda National Sero-Behavioural Survey showed that among couples where at least one partner is HIV positive, 40% are HIV discordant and only 9% are

aware of the HIV status of their spouse(66). There is also evidence, where there has been individual testing within couples, that disclosure of HIV status is either delayed for several years or never occurs for various reasons including blame and fear of domestic violence and marital disruption(67).

Summary of explanations for observed trends in HIV prevalence in the 1990s

Underlying determinants	**Direction of trend**
Marital status	decrease in age-specific prevalence of being married (women)
Migration	net outflow of HIV positive individuals
Mortality	increase among HIV positive individuals
Proximate determinants	
Age at first sex	increase in primary and secondary abstinence (teenagers)
No. of sexual partners	no clear trends
Condom use	ever use increased (all ages in males)
Condom use	at last sex with casual partner increased (women)

In summary, the increase in HIV prevalence since 2000 is not fully understood. However, improved survival due to ART, changes in sexual behaviour, and a shift in government efforts in HIV prevention towards care and treatment may have played some role, but it is difficult to establish their relative contribution. There were no changes of in and out migration among HIV positives during this period.

2.6 Limitations of using data from population cohorts to examine trends in HIV infection.

Although population cohorts such as the MRC cohort provide a unique opportunity to examine data on trends in HIV infection, they are susceptible to limitations that could limit their validity and generalisability to other populations. Firstly, there is a tendency for the participants to modify their behaviour as a result of being followed in such longitudinal studies even in the absence of any active intervention other than provision of testing and counselling, and HIV education (Hawthorne effects). Secondly, the dates of seroconversion for HIV incident cases were estimated to be the mid-point between the date of the last negative test and the first positive test. For an individual who participated in one survey round and was HIV negative but subsequently participated after three survey rounds and was found to be HIV positive, the midpoint would not necessarily reflect the actual year of seroconversion. The actual date could have been anywhere between the two survey rounds in which such an individual participated, in this case over 4 years. Thirdly, our general population cohort was carried out in one small part of Uganda, one of those that were first affected by the HIV epidemic, and it is not possible based on data from the cohort alone to determine how generalisable the findings are to other parts of the country. Finally, despite the large size of the cohort, the annual number of HIV incident cases was not sufficient to provide precise estimates of incidence or of trends in incidence, and consequently difficult to interpret. One potential way of addressing this limitation is the use of alternative statistical methods such as moving averages which smooth the data and which may provide a clearer picture of trends in HIV incidence. Such an approach could be explored in future analyses.

2.7. Explaining age and sex differences in HIV prevalence

2.7.1. Sex differences in HIV prevalence

The observed sex-differences in HIV prevalence are similar to those that have been observed in other sub Saharan African countries with women having higher rates than men. Several explanations have been suggested for these sex differences. Age at first

sex in girls is much younger than in boys, and often girls tend to have sex with older men who are likely to provide better economic support but are more likely to be HIV positive. Kelly and colleagues have demonstrated that the age difference between young women and their male partners is a significant HIV risk factor, and that this could partly explain the high HIV prevalence in young women(68). Similarly age differences between female and male sexual partners were found to be a major behavioural determinant for the more rapid rise in HIV prevalence in young women than in men in Manicaland(69).

In addition, there is evidence that male-to-female HIV transmission is more efficient than from female-to-male(65, 70). There are several biological factors that make women more susceptible to HIV than men: in the young age group the transition of the mucous membrane in young girls from a thin single layer of cells to a thick multi-layer wall is often not completed until the late teens or early twenties. The "immature" genital tract surface is thus less efficient as a barrier to HIV(71). The mucus of the female genital tract that acts as a physical barrier may also be less efficient in the presence of sexually transmitted infections. Asymptomatic and untreated STI, such as gonococcal and chlamydial infections among women are more prevalent than in men and contribute to their increased susceptibility to HIV. HSV-2 infection rates in women are much higher than in men and this also contributes to higher HIV prevalence(17). While we did not collect data on intravaginal practices such as "dry sex" and vaginal cleansing in this population, there is data indicating that this is common in different populations in sub-Saharan Africa(72) and could be other possible risk factors that may increase women's susceptibility to HIV infection. Bacterial vaginosis has also been shown to be associated with increased HIV acquisition(73). Women are also less likely than men to negotiate safer sex practices such as use of condoms(74-75).

The decline in HIV prevalence among 20-24 year old men appears to have stopped approximately three to four years earlier than in women of the same age group. The reasons for the decline in prevalence stopping earlier in young men than women may be related to the transformation of the rural study area in the mid 1990s. This area, that was

quite rural in the early years of the study turned into a "semi urban" area with more transport connecting neighbouring towns and the district capital along the trans-African highway and electrification of the trading centre in the study area. Rural men started to trade with the neighbouring towns that had much higher HIV prevalence than the rural communities. Often men travelled and occasionally stayed away from home for a few days. With a little more income at their disposal, this could have put them at higher HIV risk through sexual mixing in the urban setting with higher prevalence among women available for sex. However this "urbanization" effect on HIV prevalence could have taken a few more years to change prevalence trends in women. This lag could have been due low transmission potential of HIV from the men to their rural partners, since on average transmission will occur after many sex acts.

2.7.2. Age differences in HIV prevalence

Differences in sexual behaviour and the uptake of HIV prevention among different age groups could partly explain age differences in HIV prevalence. In the early years of the epidemic peak incidence was estimated to occur among 13-24 year olds(76) and HIV prevention control often targeted young adults and those who were unmarried. Less focus was put on people who were ever married and those aged above 30 years. In addition young adults are more likely to use condoms than older people(77), which could have contributed to the decline in prevalence among young age groups. The increase in HIV prevalence among those aged 35 years and above may be a consequence of delayed and slower sexual behavioural change in older persons, who are less likely to change their behaviour and are less amenable to safer sex interventions than younger age groups.

Another possible epidemiological reason is that different cohorts of individuals pass through different age-bands. For example the rise in HIV prevalence seen among the 35+ year age group in 2000s could be due to an earlier high prevalence among the 25-34 year olds in the 1990s. Similarly the decline among the 20-24 year olds in the 2000s could be partly a reflection of low incidence and prevalence among the 13-19 year olds in the 1990s.

2.8. Comparison with other countries in Africa

What have been the trends in HIV prevalence and incidence in other East and Southern African countries? The decline in HIV prevalence in the Uganda MRC cohort between 1990 and 2000 and indeed in other parts of the country occurred in a population that received health education through national programmes. There are a number of other African countries that also initiated national HIV health campaigns including Zimbabwe and Kenya. These are two countries where HIV prevalence has also declined.

2.8.1. Zimbabwe

In Zimbabwe data from the antenatal clinics (ANC), prevention of mother-to-child transmission (PMTCT) and VCT programmes, the ZVITAMBO clinical trial (a large vitamin study) and the Manicaland studies (from rural Zimbabwe) all indicated that there was a decline in HIV prevalence from 2000 to 2004 in both urban and rural populations. According to ANC data, HIV prevalence declined from 32.1% in 2000 to 23.9% in 2004 ($p<0.001$)(78). Similarly data from the ZVITAMBO study that enrolled pregnant and post-natal women in Harare indicated that HIV prevalence declined steadily from 36% in 1996 to approximately 21% by mid 2004. The Manicaland rural population household survey also observed a decline in prevalence among women aged 15 to 44 years from 25.9% to 22.3% ($p=0.015$). There was a corresponding decline among men between the ages of 15 and 44 years old, from 19.5% to 18.2% ($p=0.01$), with declines in all age groups except men over 35 years. Though the declining trends in the Manicaland study were significant they were much weaker (14% in women and only a 7% reduction in men) than the trends seen in Uganda between 1989 and 2000. A more recent analysis of all published and unpublished data available on trends in HIV prevalence, incidence and mortality has indicated evidence of a decline in prevalence from the late 1990s up to 2006, a rise in mortality in the 1990s that levelled off after 2000, reductions in risky sexual behaviour and a fall in HIV incidence(79).

2.8.2. Kenya

In Kenya the epidemic trends have been monitored through annual sentinel surveillance in ANCs since 1990 and sexual behaviour through the national DHS in 1989, 1993, 1998 and 2003(80). There were strong indications from the surveillance data that HIV prevalence started declining around 1998 in some of the sentinel sites and in other sites from 2000. Mean HIV prevalence in five sites declined from 25.1% in 1998 to 7.9% in 2004 (69% decline). A decline in other sites started later, prevalence falling from 14.7% in 2001 to 4.3% in 2003.

2.8.3. Explanations for trends in HIV prevalence in Kenya and Zimbabwe

A decline in HIV prevalence was seen in both Zimbabwe and Kenya over time. A review of data from Zimbabwe to explain the HIV decline indicated that behavioural changes, mainly reductions in extramarital, commercial and casual sex, made the greatest contribution. These were thought to be as a result of increased awareness due to AIDS associated mortality and economic decline resulting in less disposable income amongst men, in addition to HIV prevention programmes(81). In Zimbabwe sexual behaviour data have indicated trends towards less risky behaviour which explain the decline in HIV prevalence. For example in the eastern part of the country a delay in sexual debut among teenage boys and girls and decline in reported casual sex among sexually active men and women were noted. There was also a reported increase in consistent condom use with recent casual partners(82). Similar findings were observed using data from other surveys in the country such as the Young Adult Survey and the Population Services International survey that indicated reductions (though not statistically significant) in sexual experience among those aged 15-19 years between 2001 and 2003, and a significant decline in reported casual partners in the past 12 months(78). Similarly trends in HIV prevalence in Kenya reflected changes in sexual behaviour observed in the DHS which included a rise in age at first sex, an increase in condom use and a decrease in the proportion of adults with multiple sexual partners in the last 12 months(80).

Though there was a decline in HIV prevalence in both countries there are some differences from Uganda. First, the decline in Zimbabwe and Kenya occurred much later than in Uganda and secondly the decline was smaller than in Uganda. An older HIV epidemic in Uganda is one factor that could explain the timing of the decline in HIV prevalence. The first HIV/AIDS cases were identified in Uganda in 1982, compared with late 1987 in Kenya and the mid 1980s in Zimbabwe. Modelling work in Uganda indicates that HIV incidence peaked in 1987 or shortly thereafter and declined rapidly to about 1993 suggesting that behavioural changes had taken place by 1987 and continued at least through to 1993. In Zimbabwe HIV incidence is estimated to have peaked between 1988 and 1990 in the capital Harare, with the peak in other parts of the country occurring several years later(83). The peak incidence in Kenya is not well documented though it could have occurred around 1993(84).

One of the significant factors that could have contributed to the decline in HIV prevalence and changes in sexual behaviour in all countries was the government developing AIDS policies and establishing National AIDS Control Programmes. In both Kenya and Zimbabwe these were established in 1987 at about the same time as in Uganda.

The high-level political support accorded to the national HIV prevention efforts in Uganda early in the epidemic was not evident in Kenya until the late 1990s. There was political denial of the existence of HIV in Kenya for much of the 1980s and 1990s. Similarly the political and economic climate in Zimbabwe in the 1990s did not offer support to the prevention efforts and this could have delayed the decline in HIV prevalence.

We have seen a recent increase in HIV prevalence in Uganda but not in the other two countries. There are however data from the 2007 Kenya AIDS Indicator Survey conducted by CDC, World Health Organisation (WHO) and the Kenya Medical Research Institute indicating that HIV prevalence rose to 7.8% in 2007, a slight increase from the 6.7% prevalence recorded in 2003. No data on an increase in prevalence have

come from Zimbabwe. Whether the recent increase in HIV prevalence seen in Uganda will be observed in the other East and South African countries needs to be closely monitored.

2.9. Conclusion

Very little was known about HIV/AIDS in Uganda and in many other parts of sub Saharan Africa prior to the mid-1980s. Using data from the rural population cohort of the MRC Programme and from other sources in Uganda trends in HIV prevalence and incidence have been described. Overall, HIV prevalence declined in both men and women between 1990 and 2000 especially in those aged 20-24 years, but then increased from 2000 onwards. On the other hand, in those aged 35 years and above, a steady increase in HIV prevalence was observed in both females and males throughout the study period. I used a proximate determinants framework to better understand the trends in HIV prevalence. The decline in prevalence could have been due to the natural course of the epidemic but also due to underlying determinants (a net outflow of HIV positive migrants, more deaths of HIV positive individuals) as well as proximate determinants (reductions in risky sexual behaviour). Reasons for the increase in prevalence since 2000 are not fully understood although a shift to more risky sexual behaviour and improved survival due to ART may have played a part. Trends in HIV incidence rates are less clear than prevalence trends. The decline in incidence seen from the early 1990s could be an indication that the decline could have started earlier, possibly in the late 1980s.

Chapter 3. Interventions to prevent HIV transmission

Summary

In the early years of the HIV epidemic in Uganda, government efforts to control the epidemic were largely dependent on the ABC (Abstinence, Be faithful, Condom use) model, which led to behaviour change and a subsequent significant decline in infection rates. There was however still a need to develop and evaluate other prevention strategies to control the epidemic. A series of large clinical trials have been conducted in Uganda to evaluate potential HIV prevention strategies. Based on our knowledge of HIV transmission dynamics and associated risk factors, key interventions were designed and evaluated among established adult cohorts. The first intervention aimed to evaluate the impact of a behaviour change programme with or without improved management of sexually transmitted diseases (STDs) on HIV transmission. Subsequent interventions were based on an understanding that women are more vulnerable to HIV, and hence there was a need to evaluate female-controlled prevention methods (vaginal microbicides). Despite evidence that STDs are important cofactors in the transmission and acquisition of HIV infection and that risky sexual behaviour increases the risk of HIV, a well designed and implemented trial targeting these risk factors did not show any effect on HIV transmission. Similarly a large phase III vaginal microbicide trial that evaluated PRO 2000 vaginal microbicide gel that had shown promise in animal and in vitro studies did not prevent HIV transmission among sexually active women. Possible reasons for the lack of effect of these prevention strategies are examined.

3.1. Introduction

This chapter describes trials carried out to evaluate the impact of preventive interventions on HIV transmission in the MRC cohorts in Uganda and examines why the interventions showed no impact. The first six years of epidemiological and clinical HIV research in the MRC Uganda rural cohort provided an understanding of HIV

transmission dynamics. It was evident, as data in chapter two have shown, that HIV prevalence was high (8%) and that the epidemic was having a substantial demographic and socio-economic impact on the study population, reflecting what was happening in the whole country and possibly in many other parts of Africa. At that time the available prevention strategies were based on the Abstinence, Be faithful and Condom use (**ABC**) model. Briefly the details of the model are: Abstinence focuses mainly on young persons who have never had sex and urges them to delay starting sexual activity (primary abstinence). It also encourages secondary abstinence (returning to sexual abstinence after a period of sexual activity) among those adults not in permanent stable relationships. The 'Be faithful' component encourages individuals, after determination that none of the partners is HIV infected, to have one long-term or life-long sexual partner but also acknowledges polygamous relationships where this is within cultural and religious norms ('Zero grazing'). The Condom aspect aims to ensure consistent and correct condom use with non-regular sexual partners. To facilitate this concept there was a government commitment to ensure regular and sufficient supplies of condoms. Condom promotion and distribution was through government health facilities, high risk venues (e.g. bars and hotels), but also through social marketing. A number of approaches were used to deliver these messages such as newspapers and youth-targeted radio programmes. While there is evidence that these interventions had some impact on HIV transmission, the prevalence and incidence of infection continued at a high level, and it was important to design and evaluate new preventive interventions that could potentially achieve substantial reductions in HIV incidence.

This chapter describes two large trials conducted in Uganda. The Masaka STD/behavioural intervention trial (1994-2000) and the vaginal microbicide phase III trial (2005-2008) under the Microbicides Development Programme (MDP), were both conducted in rural Masaka district. The STD/behavioural study was a three-arm community randomized controlled trial of 18 rural communities (approximately 94,000 adults). Impact assessment was on a subset of approximately 14,500 adults. The MDP trial was a multicentre randomized double-blind study conducted in 4 African countries among sexually active HIV negative women. The trial enrolled 9385 women, 850 of

whom were recruited in Uganda. The main outcome measure in both studies was incidence of HIV infection.

3.2. STD/behavioural interventions

The predominant mode of HIV transmission among adults in sub Saharan Africa is heterosexual transmission. This is influenced by many factors including sexual behaviour and biological determinants such as the presence of sexually transmitted infections(85-86). Promotion of safer sexual behaviour and control of STDs were therefore important potential strategies to prevent HIV infection(87-88). The sexual behaviour change model used more innovative strategies and went beyond what was being provided through the national programmes. The national HIV campaign programme was formalised Information, Education and Communication (IEC) strategy. Prior to 1995 the strategy was through mass media (radio and print) and institutional communication, with virtually no personal communication about HIV/AIDS. The different organizations involved were largely faith-based, community-based, local and international non-governmental organizations in addition to government institutions. There were however some limitations to this strategy – radio and print media might not have been able to reach sufficient proportions of the population especially in rural areas due to poor coverage, timing of the broadcasts and illiteracy. Also, the institutions were likely to disseminate some but not the entire IEC package. For example the faith-based organizations were more likely to disseminate information on abstinence and faithfulness but not information on condoms. There were also limitations in government health facilities: few staff running already very busy clinics and not able to offer quality STD care, health education, counselling and testing, and with an irregular supply of condoms and STD drugs.

No randomized controlled trials had been conducted in sub-Saharan Africa to assess the impact of these prevention strategies prior to the Mwanza (Tanzania) sexually transmitted diseases (STD) intervention trial, which was conducted between 1991 and 1995. The trial targeted treatment of symptomatic STD patients using syndromic

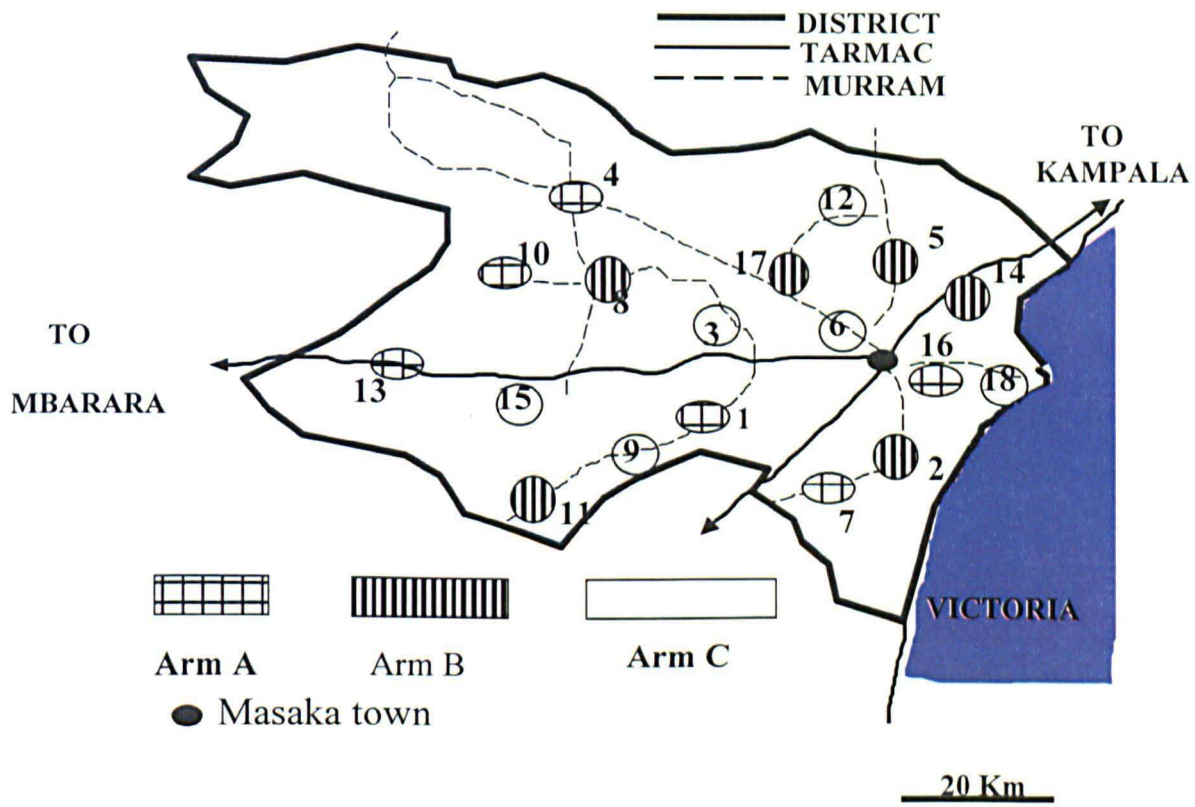
management(28). This trial was followed by the Rakai trial of STD mass treatment of the general population (1994-1998) in Uganda(29). In contrast with trials of STD treatment, no randomized trial of sexual behaviour interventions using HIV incidence as an outcome measure had been reported from Africa(89-90), even though governments and agencies spent a great deal of money and resources on health promotion and education programmes to change sexual behaviour.

The rationale of STD management as an HIV prevention method is based on STD cofactor effects on sexual HIV transmission indicating at least a two to five fold increased risk for HIV infection among persons who have other STDs, including genital ulcer disease and non-ulcerative, inflammatory STDs(31, 91). STDs facilitate HIV infectiousness and susceptibility through various biological mechanisms: inflammatory STDs such as gonococcal and chlamydial infections have been shown to increase HIV shedding and HIV viral load in genital secretions(92-93). In genital ulcerative diseases there is disruption of the epithelial and mucosal barriers which leads to increased susceptibility to HIV infection. In addition there is increased HIV shedding in the genital lesions among patients with genital ulcer disease. Correct diagnosis and early STD treatment is thus a potential HIV prevention strategy. The aim of the intervention was to reduce the prevalence of STDs in the population and thus to reduce the role of STDs in HIV transmission.

To better understand the contribution of behaviour change and improved STD management interventions to HIV prevention, a community randomized trial was designed and conducted in rural Masaka district, south west Uganda between 1994-2000. The hypothesis was that unsafe sexual behaviour and acquisition of an STD were independent risk factors for HIV infection(94). The trial aimed to assess the impact of an IEC package alone and in combination with STD management at primary health care level, and to determine the impact of each approach on HIV incidence and other STDs(21-22). The trial consisted of three arms with six communities in each arm. Six communities were allocated to receive behavioural interventions alone (group A), six communities allocated to behavioural and STD interventions (group B), and six

communities to routine government health services and community development activities (group C). The elements of randomization were communities (each with 10-12 villages) that had government health facilities. The 18 trial communities were selected and grouped according to defined characteristics: type of road passing through (a major road, secondary road or no secondary road); distance from the district capital (< 20 km, 20-40km, >40km); and quality of health facility and level of staffing (low, medium, high). To minimize contamination, the communities within each triplet as well as between triplets were as geographically distant as possible (figure 3.1).

Figure 3.1 Masaka Trial study communities



TRIPLET	ARM A	ARM B	ARM C
I	1. Kakunyu	2. Kanywa	3. Mirambi
II	4. Ssembabule	5. Kalungu	6. Kawoko
III	7. Kyantale	8. Mateete	9. Kiwangala
IV	10. Lwebitakuli	11. Ndagwe	12. Mbiriizi
V	13. Katovu	14. Mukoko	15. Kalisizo

3.2.1 Intervention activities

The IEC component of the study consisted of community-based and school-based programmes, which were conducted in two of the three study arms, in a total of 12 communities (arms A and B). The IEC package was based on the Behavioural Change for Interventions model (BCI) which focuses on knowledge acquisition, skills development, motivational support and attitudes development. The aim was to give correct knowledge, to correct misconceptions about HIV and other STDs, and to promote safer sexual behaviour and practices. The community-based intervention was implemented using locally accepted community approaches such as drama groups with particular attention to development of peer-based education systems including establishing AIDS prevention committees, community educators and a parish coordinator in each community. The interventions were implemented using various channels that included small and large community meetings, drama, video shows focusing on specific HIV messages, and distribution of information leaflets and one-one discussions. The central IEC staff (coordinator, health educators, condom distributor) oversaw the community-based activities through training and supervision of field based groups whose main tasks included providing information and advice about HIV/AIDS and STDs. They also carried out condom promotion and distribution. A total of 540 field based workers implemented the activities.

The community-based IEC channels were carefully chosen so as to be relevant to the target population. For example, drama shows are a familiar and acceptable channel to communicate a story in such settings. Five plays were written in the local language by a consultant and covered important messages: the role of sugar daddies, extra marital sex, the role of peer pressure among youths especially girls, and community resistance to condoms. The video shows were an innovation that excited and attracted large audiences and showed the local plays in addition to covering other topics: disbelief about AIDS and subsequent sex with AIDS widows, counselling and testing, sex and consequences among school youths. The intervention was adequately delivered with an

average of six attendances of IEC activities (video shows, drama shows, health education by field-based community educators) per adult target(95).

The school-based programme was a 19-activity extracurricular school-based comprehensive AIDS education programme that targeted students in the top two years of primary school and the lower two years of secondary school in schools from the IEC communities. A senior woman teacher and a science teacher in each school were identified and requested to take part in a series of three training workshops prior to embarking on implementation in their various schools, and within the school's normal timetable at the teachers' discretion. They would either be incorporated into existing lessons or taught during out-of-school hours.

The school-based programme was initiated by the trial team as AIDS education in Uganda was quite limited in both content and coverage. The programme was adapted from the WHO/UNESCO' School Health Education to prevent AIDS and STD- A Resource Package for Curriculum Partners(96). Though this was a comprehensive teacher-based intervention that focussed on basic HIV/AIDS information, role-play activities, condoms and safe sex negotiation, there were some limitations of the programme. We purposely left the implementation of the programme to the discretion of the teachers either to be incorporated into existing lessons or taught during out of school periods, which could potentially impact on its quality. We were also aware that there could be resistance to condom discussion in schools either from the teachers themselves or from the wider community including parents. The trial team made support visits to the schools that aimed to discuss with teachers any difficulties with the intervention rather than supervise how they were actually delivering the activities in classrooms. We utilised both qualitative and quantitative techniques to evaluate the programme. In summary, we noted that the programme was incompletely implemented and some topics such as condoms and role-play activities were hardly covered.

The STD intervention targeted the six communities in arm B (approximately 32 000 adults) and involved the participation of both government and private health units. The

aim was to improve STD management by focusing on the quality of care and followed the syndromic management approach(97). STD syndromic management, recommended by WHO, is a cost-effective strategy based on treatment of patients with defined clinical syndromes for the major aetiologies shown to be responsible for these syndromes in the local population. This approach is appropriate in developing countries with limited laboratory infrastructure as it avoids the need for patients to be tested for specific aetiologies at the point of care. Health workers from all units were trained on correct syndromic diagnosis and treatment, counselling and future risk reduction, condom use, follow up visits while on treatment, partner notification, and record keeping. Regular supervision by trial central staff was carried out every 2 weeks. All units were supplied with basic equipment and supplies including condoms and essential drugs to facilitate improved STD management. We ensured that health units had sufficient stocks of essential drugs and supplies at all times.

3.2.2. Comparison arm activities

Activities in the comparison arm (arm C) focused on improving community development and general health-related issues important to and chosen by communities. Three main areas were identified in consultation with the community. Firstly, support and training to existing profit making and self-support groups and clubs, in management skills and appropriate technology to generate income and become self-sustaining. Secondly, a home-based care programme to treat elderly and bedridden clients. Thirdly, health promotion targeting important topics such as malaria, breast feeding practices, family planning, sanitation and diarrhoeal diseases.

All study arms were provided with social marketing of condoms and voluntary HIV counselling and testing.

3.2.3. Impact evaluation and analysis

Impact assessment of the interventions was through three Knowledge, Attitude, Behaviour, Practice (KABP) and serological surveys, conducted at 18–24 month intervals, in a sample of 750–1000 adults (13+ years) in each community. The sample

was obtained by surveying 3–5 villages closest to the health unit. The primary outcome was HIV incidence. Secondary outcomes were incidence of herpes simplex virus type 2 (HSV2) infection and active syphilis and prevalence of gonorrhoea, chlamydia, reported genital ulcers, reported genital discharge, and markers of behavioural change. Analyses were performed separately comparing arms A vs C and B vs C, taking account of the matching of communities, and potential confounders including baseline HIV prevalence.

3.2.4. Summary of results

Baseline results: Overall the baseline enrolment was high (72% of eligible population). Despite grouping and matching study communities based on HIV-related community characteristics (type of main roads, distance from the district capital, the quality of health facilities and level of staffing), HIV baseline prevalence varied by study communities (range = 4.2-20.3%) and also between arms; 8.8% in arm A, 10.1% in arm B and 10.4% in arm C. There was however fair baseline comparability between study arms for potential confounders for HIV and STD infections except religion and history of genital ulcers which varied substantially. Reported rates of ever use of condoms, use with last casual partner, and proportion reporting two or more sexual partners in the past year were similar in all arms. The prevalence rates of STDs (syphilis, HSV-2, gonorrhoea and chlamydia) were also fairly similar across study arms. All analyses were adjusted for age, sex, and community-level baseline prevalence of the outcome (HIV, HSV2, syphilis, reported genital ulcers, reported genital discharge).

Intervention impact results: The proportions of eligible individuals providing a blood sample were 72% in the baseline survey, 72% in the round 2 survey and 91% in the round 3 survey. The median follow up of communities was 3.8 years (range 3.2-5.0). A total of 304 HIV events occurred over 41,060 person years at risk, with overall unadjusted incidence rate of 0.74 per 100 person years at risk. Highest incidence was observed in communities with highest baseline prevalence. Incidence of HIV did not differ between groups A and C or between groups B and C, even after adjustment for age, sex, and baseline HIV prevalence (table 3.1), or after additional adjustment for

genital ulcer disease and religion (group A *vs* C, incidence rate ratio 0·94 [95% CI 0·60–1·45], p=0·72; group B *vs* C 1·00 [0·63–1·58], p=0·98).

Impact on STDs was observed on HSV-2 incidence – lower in group A (behavioural intervention alone) than in group C (comparison arm) with an incidence rate ratio of 0.65 (95% CI 0.43-0.97). This effect remained significant after additional adjustment for religion and genital ulcer disease. However there was no effect in group B (the same behavioural intervention plus STD intervention). There was an impact on active syphilis (high RPR titre) and prevalence of gonorrhoea in group B compared to group C (active syphilis incidence rate ratio, 0.52 [0.27-0.98]; gonorrhoea prevalence ratio, 0.25 [0.10-0.64]).

Table 3.1 HIV prevalence, incidence and incidence rate ratios by triplet and arm

Triplet	Baseline Prevalence (%)			HIV incidence/100 pyar (number of events/pyar)			Incidence Rate Ratios (95% CI)			
	Trial Arm			Trial Arm			A vs C		B vs C	
	A	B	C	A	B	C	Unadjusted	Adjusted *	Unadjusted	Adjusted *
1	10	9	7	0.5 (14/2716)	0.8 (19/2529)	0.3 (9/2625)	1.50	0.96	2.19	2.01
2	10	11	9	1.2 (20/1627)	0.5 (12/2336)	0.6 (16/2596)	1.99	1.40	0.83	0.62
3	11	17	12	0.8 (18/2382)	1.7 (31/1819)	1.2 (23/1888)	0.62	0.71	1.40	0.98
4	4	8	7	0.4 (6/1681)	0.5 (11/2276	0.5 (15/2943)	0.70	0.97	0.95	0.87
5	5	9	8	0.3 (9/2768)	0.4 (8/1975)	0.9 (21/2359)	0.37	0.51	0.46	0.54
6	11	8	20	0.6 (14/2173)	1.1 (29/2701)	1.7 (29/1670)	0.37	1.29	0.62	0.97
Overall	9	10	10	0.61 (81/13346)	0.81 (110/13634)	0.80 (113/14080)	0.75 (0.36-1.57) p=0.36	0.92 (0.62-1.37) p=0.61	0.94 (0.52-1.70) p=0.70	0.91 (0.56-1.47) p=0.41

p-value is for paired t-test;
* Adjusted for age, sex, baseline HIV prevalence.
pyar: person years at risk

Table 3.2 HSV-2 and syphilis incidence and incidence rate ratios, and gonorrhoea and chlamydia prevalence and prevalence ratios

Trial Arm			Rate Ratios (95% CI)			
A	B	C	A vs C		B vs C	
HSV-2 incidence/100 pyar (events/pyar)†			**Unadjusted**	**Adjusted ***	**Unadjusted**	**Adjusted ***
2.3 (100/4381.6)	3.6 (167/4595.5)	3.5 (161/4628.6)	0.66 (0.33-1.29) p=0.17	0.65 (0.43-0.97) p=0.040	1.02 (0.59-1.74) p=0.94	1.02 (0.69-1.50) p=0.90
Active syphilis incidence/100 pyar (any RPR titre) (events/pyar)‡						
2.9 (341/11866.5)	2.1 (255/12379.7)	2.9 (366/12444.9)	1.00 (0.61-1.64) p=0.99	1.13 (0.70-1.82) p=0.54	0.71 (0.52-0.99) p=0.045	0.81 (0.64-1.04) p=0.080
Active syphilis incidence/100 pyar (RPR titre ≥1:8 (events/pyar)‡						
0.5 (72/1139.1)	0.3 (43/1449.5)	0.6 (83/1484.5)	0.90 (0.44-1.86) p=0.73	1.13 (0.61-2.07) p=0.64	0.53 (0.33-0.84) p=0.016	0.58 (0.35-0.96) p=0.039
†Gonorrhoea prevalence (%)§						
1.02 (34/3339)	0.51 (18/3540)	1.17 (42/3589)	0.75 (0.30-1.85) p=0.45	0.74 (0.30-1.82) p=0.43	0.29 (0.12-0.71) p=0.016	0.28 (0.11-0.70) p=0.016
†Chlamydia prevalence (%)§						
1.35 (45/3339)	1.92 (68/3540)	1.81 (65/3589)	0.59 (0.20-1.72) p=0.26	0.59 (0.20-1.72) p=0.26	0.99 (0.70-1.41) p=0.94	0.99 (0.71-1.39) p=0.97

* Adjusted for Age and Sex. †Also adjusted for baseline HSV-2 prevalence.
‡Also adjusted for baseline RPR prevalence.
§ Pooled for R2 and R3
Pyar – person years at risk

3.3. Why was there no reduction in HIV incidence in the STD/behavioural trial?

It is surprising that the interventions did not have an effect on HIV incidence, despite good evidence that HIV is transmitted through risky sexual behaviour and facilitated by the presence of STDs. Many HIV intervention trials conducted to date have shown negative results(98). In general, there are several reasons why interventions sometimes do not have the anticipated impact and some of these could have played a role in this trial. A recently proposed framework to discuss the negative results of randomized controlled trials of HIV prevention sets out the following potential explanations for such findings(98-99): (i) the concept is wrong; (ii) the concept is right but the actual intervention is "inert" or insufficient to demonstrate impact; and finally (iii) there were problems with the design or conduct of the trial. Following this framework, possible reasons for the findings in this trial are explored.

3.3.1. The concept was wrong

Prior studies in this rural district had indicated that STDs were highly prevalent, up to 12% for syphilis and up to 36% in males and 72% in females for HSV-2(17, 100). Similarly, baseline STD prevalence and reported STD symptoms in the trial communities were high. Baseline data also indicated high risk sexual behaviour such as low condom use, high numbers of sex partners and casual sex in the last year. About 6% reported age at sexual debut below 13 years and approximately 40% between 16 and 19 years(21). The MRC trial included a population where the predominant mode of transmission is heterosexual. The interventions that targeted sexual behaviour change and improved management of STDs were thus appropriate and had the potential to have an impact on HIV transmission.

The IEC intervention used locally appropriate channels and was largely dependent on the IEC team who were residents within the communities throughout the entire trial

period with the aim of creating a sustainable and replicable IEC strategy. The school-based component of the IEC was implemented as best as was practical given available resources although we have noted its limitations. The improved STD management was through syndromic management, a relatively simple approach to incorporate into the existing health care infrastructure. We focused on the factors that affect the quality of care in both government and private health units with regular support and supervision. Thus there is good evidence to suggest that the concept was right but the actual intervention could have been insufficient to demonstrate an impact at population level.

3.3.2. The concept is right but the actual intervention is "inert" or insufficient to demonstrate impact

The interventions were carefully designed and appropriate for the community and were of high quality(21). The behavioural intervention was largely delivered by approximately 560 field-based workers who had received very intensive training and were regularly supervised by central staff. There were high levels of behavioural intervention activity in the twelve communities with excellent coverage rates. For example, over the five year intervention period there were approximately 390,000 individual attendances at over 80,000 IEC activities (community meetings, video shows, drama shows, and individual meetings with community educators), representing a mean of 6 attendances for each of the 64,000 target adults in the behavioural intervention communities with similar attendance rates for men and women. Approximately 58% of the attendances were among those aged 13-24 years and 42% among those aged above 24 years. A total of 164,063 information leaflets were distributed. This is a mean of 2.6 leaflets per target individual. Many leaflets would have been read by more than one individual. We also demonstrated that the messages were understood and retained by the participants(95, 101). Across all channels, many people were able to remember the main message three months or more after exposure. Individuals were most likely to remember the message from a community educator, followed by drama/video and then leaflets. Similarly the trial appeared to be acceptable to the community and in particular free HIV testing and counselling were very much appreciated. The field workers built their skills

and confidence and were well respected and accepted by their communities as providers of HIV/AIDS information(102).

On the other hand there was evidence that the school-based component of the IEC intervention was incompletely implemented due to insufficient classroom time and some topics such as condoms not sufficiently covered due to fear and sensitivity of the subject in such communities. These observations contrast with those observed in some other studies that have evaluated the impact of sex and HIV education programmes in schools. A review by Kirby and colleagues of 83 studies that measured the impact of curriculum-based sex and HIV education programmes on sexual behaviour and mediating factors in a wide variety of countries, cultures, and groups of youth showed that the majority of studies found a significant positive impact on sex behaviours or outcomes(103). An analysis of the curricula that had a positive impact identified 17 common characteristics that included their development, content and implementation. There are two possible reasons for our negative findings. First our programme was not adequately implemented because of insufficient classroom time to cover non-examinable topics, fear of discussing condoms that were not fully supported by parents and wider communities, and unfamiliarity with role-play techniques among teachers. Secondly, the programme was an adapted version of the WHO/UNESCO's school curriculum that had not been previously adapted, and may not have been appropriate for the target group in our settings. We also did not adequately assess the needs of our target group and consequently some of the activities were not consistent with the community expectations. It is therefore evident that our school-based programme did not fully comply with the Kirby characteristics.

Interestingly the community development activities in the comparison group were also equally well accepted as the population regarded development as more important than AIDS education. STD treatment interventions were also adequately delivered in the six intervention communities. Over 12,000 STD cases (65% females) were seen at both the government and private health units; and approximately three-quarters of cases were in

those aged 20-39 years(21). Therefore it does appear that the interventions were well delivered but were "inert" because they were applied at the wrong stage of the epidemic and in a population where STD rates and risk behaviour had already decreased. This was confirmed by the comparative analyses and modelling of the three STD trials discussed in section 3.3.4.

A sub analysis examining outcome measures at an individual level was conducted comparing the arm that received the behavioural intervention alone, without improved STI management (arm A) and the comparison arm (arm C). Arm B was excluded as the effect of the behavioural intervention may be different in the presence of the STD intervention. This analysis differed from that of the main trial analyses that were performed at a community level(23). HIV incidence rates were lower in those who reported attending IEC activities in the past year compared to those who did not. This effect was significant in women who attended any activity (p=0.024) and in men who attended community meetings (p =0.045). This observed effect on HIV was examined to see whether it was biased by non-attendance as a result of being away (a proxy for risky sexual behaviour). The effect was unchanged when adjusted further for confounding factors (age group, marital status, religion and travel outside the district). One limitation of this analysis could have been that measurement of IEC attendance and HIV incidence was done in the same surveys (rounds 2 and 3), making it difficult to tease out whether HIV sero-conversion occurred before or after attending the IEC activity. However a similar effect was observed when we restricted analysis to attendance of IEC activities reported at round 2 and sero-conversion between rounds 2 and 3. Attendance of IEC activities was however not associated with a reduction in sexual behaviour (reported sexual partners and condom use). This could be due to reporting bias as in many other studies(104-105), relatively small numbers of individuals engaged in high-risk sexual behaviour in the entire community so that it was difficult to detect any changes, or the methodology used (KABP questionnaire) which may not have been sufficiently accurate to measure sexual behaviour changes. This was an observational analysis and associations may have been due to confounding. For example, those accessing the

interventions may have differed from other community members, and may have been at lower risk of HIV even in the absence of the intervention.

3.3.3. There were problems with the design or conduct of the trial

The community development activities in the comparison arm, such as support to existing groups and clubs, home based care for the elderly and bedridden clients, and health promotion on non-HIV health problems are unlikely to have had an impact on the outcome measures (HIV incidence and STDs). It is however plausible that the presence of our own field staff from an HIV research organization, and social marketing of condoms and voluntary HIV counselling and testing as well as serological surveys (for which consent for HIV testing was first sought) could all have had some effect on the risk of HIV infection. If this is the case then this could potentially have diluted the true effect of the interventions. Did the intervention activities spill-over into the comparison communities? There was minimal spill over from the STD intervention communities to the comparison and the IEC arms. We regularly assessed the attendance in the STD registers within the six STD intervention communities. Overall only 1% of all clinical visits in the STD registers within the STD communities were individuals from either the comparison or IEC-only communities, an indication that the majority of care was sought from health facilities within their own communities. There was however some spill over of IEC activities to the comparison arm - about 9% of individuals in the comparison arm reported attending at least one IEC activity, mainly attending a community meeting. Though some of this could be due to misreporting, for example reporting non-intervention mobilization meetings for serological surveys, we noted some attendance from the comparison communities was a result of insufficient geographic separation of the intervention and comparison communities.

The study might not have been adequately powered as the observed HIV incidence (0.6-0.8%, table 3.1) was lower than had been initially assumed (a background annual incidence of 1.5%, at the time of the study design).

Finally, we believe that the timing of the trial could also have played a role in the negative result. The study was initiated in 1994 and completed in 2000. As discussed in chapter two, HIV prevalence rates started declining in 1993/94 and this continued to 2000/01, with evidence of a decline in risky sexual behaviour in the late 1990's in a neighbouring study population in the same district. These declines occurred in the absence of any active intervention and were a reflection of what was probably happening in the rest of the country. This was consistent with the stage of the epidemic that was mature and generalised as has been observed in other settings with indications that reduction in risky sexual behaviour is a factor driving reduction in HIV incidence(106). The spill-over to the comparison communities, the timing of the trial in relation to the stage of the epidemic in the country, and the lower HIV incidence than originally estimated suggest that the negative findings could have been partly due to problems with the study design, as well as deficiencies in the school based component of the IEC intervention.

3.3.4. Comparison of trial results with Mwanza syndromic STD management trial and Rakai STD mass treatment

The Masaka trial results in Uganda provided contrasting findings to the Mwanza syndromic STD intervention trial in Tanzania that had demonstrated an approximately 40% reduction in HIV incidence (incidence RR 0.62, 95% C1 0.45-0.85). The findings in the Masaka trial were however similar to those of the Rakai trial, also in Uganda, that evaluated the impact on HIV incidence of mass STD treatment at 10-monthly intervals and syndromic management at the time of the surveys (incidence RR 0.97, 95% CI 0.81-1.61).

In an attempt to explain these contrasting results from the three East African randomized trials targeting STDs to prevent HIV transmission, a collaborative modelling project using *STDSIM*, a stochastic simulation model of HIV and STD transmission, was conducted. The aim was to re-analyse and compare empirical data from the three trials and fit the *STDSIM* model to empirical data from each trial population, simulate

different interventions in each population and assess the relative effectiveness of different interventions in different populations. The main findings of this modelling project were that differences between the trial results could largely be explained by the differences between the three study populations(39, 107-108). Briefly, the demographic characteristics across the three trial populations were generally similar. The baseline HIV prevalence rates were however much higher in Uganda (16.5% in Rakai; 12.1% in Masaka) than in Mwanza (3.8%). The Ugandan epidemic was more mature than in Tanzania and was characterised by a reduction in risky sexual behaviour and lower rates of curable STDs at the time of the trials (Figure 3.2a and Figure 3.2b). This could have been due to the intensive national AIDS campaigns leading to high HIV awareness in Uganda, but also due to fear from the high mortality that had occurred in Masaka and Rakai in the 1980s. There were higher levels of reported sexual risk behaviour, younger age of sexual debut, and higher rates of curable STDs (gonorrhoea, chlamydia, trichmoniasis and active high-titre syphilis) in Mwanza than in the Ugandan trials except for *Chlamydia* in Ugandan men. The modelling work showed that the observed impact on HIV in the three trials was consistent with what would be expected based on the different risk behaviours and STD rates in the three trial populations. The population differences were able to largely explain the observed differences in impact(108).

In Masaka there was a reduction in HIV transmission already occurring in this setting(18) and during the trial we noted substantial secular changes towards safer sexual behaviour in the whole study population, unrelated to the main trial interventions, possibly due the nationwide prevention messages through the media and from other agencies. We reported from the same population that a substantial proportion (89%) of those who could change their behaviour had already done so(109). It is possible that even well designed and conducted IEC/STD interventions would have little chance of demonstrating a change in sexual behaviour in the presence of pre-existing background changes in a mature epidemic as in the Masaka trial population.

In summary the Mwanza trial in Tanzania demonstrated the concept of syndromic STD management in the control of HIV transmission. The lack of impact on HIV incidence in

the Masaka trial was due to interventions not being applied to the right population at the right time. The Ugandan epidemic was mature, characterised by an existing reduction in risky sexual behaviour and lower rates of curable STDs at the time of the trial. In mature epidemics a larger proportion of new HIV infections would tend to occur in stable sexual partnerships in which STD rates are lower. When STD rates are lower the biological mechanisms through which STDs affect both the infectiousness of HIV (increase in HIV shedding) and susceptibility to HIV (increase in HIV susceptible inflammatory cells and disruption of the mucosal barriers) are likely to play less a role in HIV transmission.

Figure 3.2a Proportion (%) reporting two or more sex partners in the past year by age and sex

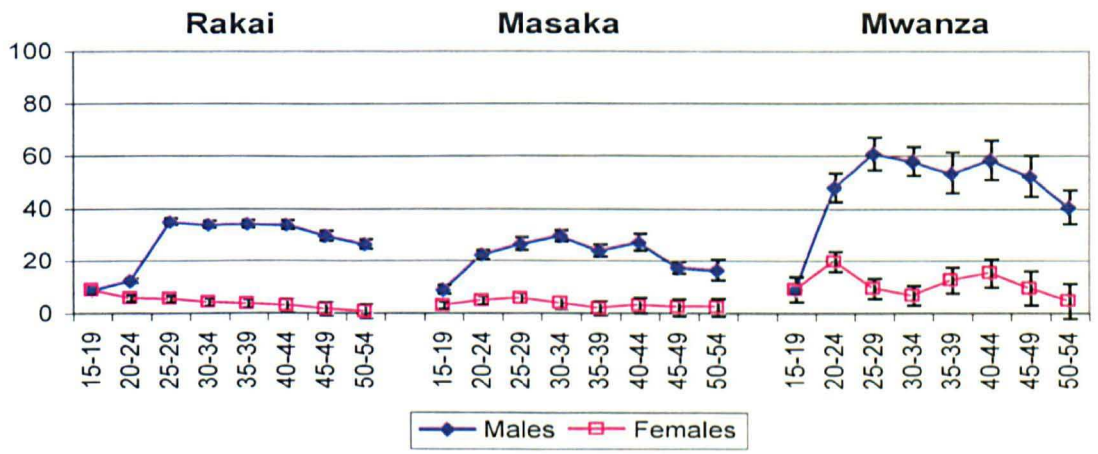
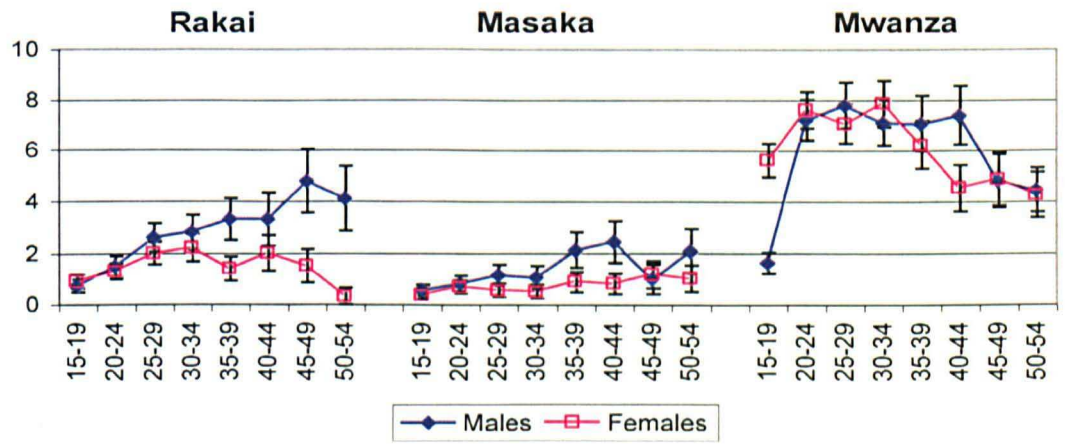


Figure 3.2b Prevalence (%) of high-titre serological syphilis (TPHA+/RPR ≥1:8 and sex at the start of the trials



Overall, we conclude that the concept of the interventions was right and the interventions were well-delivered but were "inert" because they were applied at the wrong stage of the epidemic and in a population where STD rates and risk behaviour had already decreased to a lower level. The design and conduct of the trial may also have affected the outcome as may the "sufficiency" of the interventions particularly the school-based programme of the IEC-component.

3.4. Research on vaginal microbicides for HIV prevention

Though HIV is predominantly transmitted through heterosexual intercourse in sub-Saharan Africa, women are more vulnerable for several reasons including greater genital tissue exposure, socio-economic circumstances, and limited opportunity for negotiating safer sexual practices including condom use(110-111). There is growing interest in developing and evaluating female-initiated HIV prevention methods that are not heavily dependent on partner negotiation. This includes the development of vaginal microbicides, a potential new female-controlled HIV prevention tool(112). There are various microbicide formulations, including vaginal gels, pessaries, film, and vaginal rings that release the active ingredient over time. Of the formulations that have been evaluated most are gels.

In the last decade two generations of microbicides have been tested for safety and acceptability through various phases (I and IIa) and a few have gone through phase IIb and III safety and efficacy clinical trials. The first generation microbicides were surfactants such as Nonoxynol-9 and Savvy that disrupt microbial cell membranes, thereby inactivating or killing the virus; and the vaginal defense enhancers that maintain or boost the naturally protective acidity of the vagina such as BufferGel(113). The second generation microbicides were entry inhibitors that block cellular receptors and prevent HIV from attaching to and infecting target cells. These include PRO2000, Carraguard, and cellulose sulphate(113). More recently, third generation products have been developed. These are ARV-based microbicides (tenofovir gel, UC-781 and dapivirine) that prevent HIV from replicating once inside a cell(114). Unfortunately all

first and second generation products so far tested have not shown efficacy against HIV transmission. Encouragingly, the first Phase IIb of a third generation microbicide, tenofovir 1% gel, has shown efficacy (CAPRISA 004 trial)(37).

3.4.1. Microbicide phase II trial (safety and acceptability)

We set out to establish suitable cohorts for evaluating potential microbicides in Uganda as described in chapter one through safety and acceptability (phase II), and safety and efficacy (phase III) clinical trials. After the failure of Nonoxynol-9(115), a number of trials have been conducted to evaluate promising products including SAVVY(116), cellulose sulphate(117) and Carraguard(118). We evaluated PRO 2000 gel which is a microbicide polyanionic naphthalene sulphonate polymer that blocks infection with HIV, HSV-2, *Neisseria gonorrheae*, and *Chlamydia trachomatis*(119). A phase II trial was conducted among sexually active women aged 18-45 years in Kampala, Uganda to evaluate the safety and acceptability of two concentrations of PRO 2000 gel (0.5% and 2%). After informed consent, women were randomized to 0.5% or 2% PRO 2000 gel or to a control arm (condoms only). Women in the gel arms were requested to use one applicator (approximately 2g of gel) intravaginally twice a day for 28 days. Follow-up visits were fortnightly up to 6 weeks from enrolment. The main findings were that both concentrations were found to be safe, acceptable and well tolerated(120). In the light of these findings together with early safety studies elsewhere showing the safety and tolerability of PRO 2000(121-123), and promising in vitro and animal data, it was considered justifiable to evaluate the two concentrations in a large scale phase III efficacy trial.

3.4.2. Phase III safety and efficacy trial

A phase III randomized, double-blind trial to assess the efficacy and safety of 0.5% and 2% PRO 2000 gels compared with a placebo gel for the prevention of vaginally acquired HIV infection was conducted in 4 sub-Saharan countries (South Africa, Zambia, Tanzania and Uganda) under the Microbicides Development Programme (MDP). The trial (MDP 301) was conducted between October 2005 and September 2009. Briefly, the trial enrolled sexually active HIV-negative women who were willing to give informed

consent, to have regular genital examinations and pregnancy tests, and to use the gel as per instructions. Out of 9385 women enrolled in the trial, 840 were enrolled in Uganda with a mean age of 32 years. The women were randomized to 0.5%, 2% and placebo in 1:1:1 ratio and followed for 52 weeks (up to 104 weeks in Uganda). The trial also conducted social science research to evaluate gel acceptability and barriers to adherence. The primary outcome measures were HIV incidence, and grade 3 (severe) or 4 (life-threatening) adverse events. The secondary outcome measures were HSV-2, *Neisseria gonorrheae* and *Chlamyida trachomatis* infections. In Uganda, the trial population was HIV-negative women living in an HIV discordant relationship, a study population in whom we had demonstrated good follow-up rates (11% loss to follow-up at one year) and an HIV incidence rate of approximately 12 per 100 person years of observation.

The trial was monitored by an independent data safety monitoring committee (IDMC). In February, 2008 the committee recommended the discontinuation of the 2% gel arm as there was no more than a small chance of demonstrating benefit of that concentration. However women continued to be allocated to 0.5% gel or placebo. The retention at 52 weeks of follow up was high - over 80% in all trial centres except in one centre with follow up of 75%. Overall the gel was discontinued in 17% of women largely because of the withdrawal of 2% gel following the IDMC decision, and following pregnancy. The primary efficacy analysis included all HIV-negative women after enrolment and with follow-up data, and censored at 52 weeks of follow-up or pregnancy. A secondary efficacy analysis was done with the same criteria as the primary efficacy analysis, but without censoring for pregnancy and using all follow-up data.

3.4.3. Summary of results

The main trial results indicated that the gels were not effective in preventing HIV transmission. HIV incidence at the end of the trial was 4.5 per 100 woman-years (95% CI 3.8-5.4) in the 0.5% gel arm and 4.3 (95% CI 3.6-5.2) in the placebo arm, with a hazard ratio of 1.05 (0.82-1.34). Similarly, there was no difference in incidence between trial arms when the efficacy analysis was done without censoring for pregnancy and including all follow-up data (table 3.3 At the time when the 2% gel arm was

discontinued HIV incidence was 4.7 (95% CI 3.8-5.8) in the 2% arm, 3.9 (95% CI 3.0-4.9) in the 0.5% arm and 3.9 (95% CI 3.1-5.0) in the placebo gel arm. There was no difference in the incidence of primary safety endpoints between the trial arms(25). Early clinical studies and a phase IIb trial (HPTN 035) had shown encouraging results that PRO 2000 gel would have an effect on HIV transmission in humans. The HPN035 trial that evaluated two gels (PRO 2000 gel and buffer gel) showed that PRO 2000 was associated with a 30% reduction in HIV transmission though this was not statistically significant, whereas buffer gel had no effect. However, in the larger MDP trial, PRO2000 showed no significant effect.

Table 3.3 Results of MDP301 trial of PRO 2000: HIV incidence

	0.5% PRO2000 (n=3326)	**Placebo (n=3325)**
Primary efficacy analysis*		
Participants	3156	3112
Woman-years of follow-up	2873	2836
Seroconversions	130	123
Incidence	4.5 (3.8-5.4)	4.3 (3.6-5.2)
Hazard ratio	1.05 (0.82-1.34)	1
p value	0.71	
Secondary efficacy analysis±		
Participants	3156	3112
Woman-years of follow-up	3133	3099
Seroconversions	145	143
Incidence	4.6 (3.9-5.4)	4.6 (3.9-5.4)
Hazard ratio	1.00(0.79-1.26)	1
p value	0.99	

* Efficacy analysis censoring at 52 weeks of follow-up and pregnancy

± Efficacy analysis without censoring for pregnancy and using all follow-up data

3.5. Why was there no reduction in HIV incidence in the MDP 301 trial?

I have used the framework set out in section 3.3 to try to explain the lack of effect of PRO 2000 in the MDP 301 trial. That is (i) the concept is wrong; (ii) the concept is right but the actual intervention is "inert" or insufficient to demonstrate impact; and finally (iii) there were problems with the design or conduct of the trial.

3.5.1 Was the concept of a microbicide gel to prevent HIV acquisition wrong?

There are various mechanisms of action through which microbicides would prevent HIV acquisition. The first and second generation microbicides were meant to act through disrupting the viral cell membranes (N-9, Saavy), enhancing vaginal defences (Buffer Gel) or blocking the HIV-target cell interactions (Carraguard, Cellulose Sulfate, PRO2000). None of these products however, were able to demonstrate efficacy. Only recently has the concept of a microbicide in preventing HIV acquisition been proven in the CAPRISA 004 trial using tenofovir gel(37), the mechanism of which is through inhibiting HIV replication inside the target cells. Although further confirmatory trials are needed, this has provided evidence that the concept is right.

3.5.2. Is the concept right but the actual intervention (PRO 200 gel) "inert"?

The PRO 2000 gel tested in this trial was demonstrated to have anti-HIV activity in pre-clinical studies. In these animal studies however HIV is introduced in the absence of physical sexual activity and hence there is no contact with seminal fluid. This is a different environment from that in clinical trials in humans, in which the product molecules are acting in the presence of female genital secretions, semen and physical coital activity. Some studies have indicated reduced antiviral activity of microbicides in the presence of seminal plasma(124-125). More recently, Keller and colleagues(126) have demonstrated that there is significantly diminished antiviral activity (pharmacodynamics - PD) as well as lower concentrations (pharmacokinetics - PK) of PRO 2000 gel following coitus. The mechanisms of this are not fully understood but it is possible that seminal proteins may inhibit the binding of PRO 2000 molecules to the

target cells. Though Keller's study was small and based on a single-dose design with no placebo arm it offers a potential explanation for the lack of efficacy in the PRO 2000 gel trials and calls for conducting post-coital PD and PK studies in early clinical trials prior to designing large phase III trials. It thus appears that the particular product used in this trial (PRO 2000 gel) was "inert".

3.5.3. Were there problems with the design or conduct of the trial?

The trial was carefully designed to ensure adequate power taking into account loss to follow-up and censoring of women who became pregnant during the trial(25). Poor acceptability and adherence to product use could be one possible explanation, as these could affect potential effectiveness of the product. However, the gel was reported to be highly acceptable to both women and men, who often reported that it increased sexual pleasure(127). In a number of instances women were very unhappy to be withdrawn from gel at the end of their participation in the trial because it was felt that this would decrease their sexual pleasure. Equally important is the accurate measurement of adherence to gel use(128-129). There are a number of ways in which this could be measured such as self reporting through individual questionnaire interviews, in-depth interviews and coital diaries. There are also other more independent approaches, for example counts of used and returned gel applicators, and measuring biomarkers of the product in the body or on the returned applicators. The MDP trial had a social science component that collected relevant data to help in the interpretation of results. Adherence was measured using mixed methods that included clinic interviews, in-depth interviews, and coital diaries and counting the number of used applicators returned. The data were subsequently triangulated which is a method of combining results from different methods in an attempt to get a more accurate result(129). The triangulated data indicated that the adherence in this trial was high with no differences between the trial arms. The overall reported rate of gel use with and without condom use at the last sex act was approximately 90% at all visits. Hence non-adherence was unlikely to explain the lack of effect on HIV transmission(128).

The product was designed to prevent vaginal acquisition of HIV and so if there was a significant frequency of anal sexual intercourse then the gel protection would have been less effective. Frequency of anal sex was assessed during the trial through individual interviews and focus group discussions. Only 2% of women reported anal sex in the trial, the majority of whom were from South Africa research centres. This low rate of anal sex is therefore unlikely to have affected the efficacy of PRO 2000 gel. There do not appear to have been major problems with the design of the trial.

Overall, it seems that the concept of a microbicide is right and that the MDP 301 trial was well designed and conducted. However the particular product used for this intervention (PRO 2000 gel) was "inert".

3.6. Conclusion

Two large HIV intervention trials evaluating STD management in combination with behavioural change among Ugandan adults, and a vaginal microbicide gel among high risk HIV-negative women in four African countries, did not show any effect on HIV transmission. To explain these findings, a conceptual framework for discussing negative results from randomized controlled trials of HIV prevention has been used. That is (i) the concept is wrong; (ii) the concept is right but the actual intervention is "inert" or insufficient to demonstrate impact; (iii) there were problems with the design or conduct of the trial.

The concepts of syndromic STD management and of a microbicide gel for the control of HIV transmission and acquisition were demonstrated by the Mwanza STD trial in Tanzania and the CAPRISA004 microbicide trial in South Africa, respectively. In both trials it appears that the explanation for the negative results is that the concept is right but the actual intervention was "inert" or insufficient to demonstrate impact. In the Masaka trial, the intervention was applied at the wrong stage of the epidemic in a population where STD rates and risk behaviour had already decreased. There was also evidence that there were deficiencies in the design and delivery of the school-based

component that did not fully comply with common characteristics of school programmes that have demonstrated positive impact in other settings.

Despite these negative findings there are important lessons to be learnt from these and similar trials conducted elsewhere, which can inform future HIV prevention research. The Masaka trial results together with data analysis from two other STD intervention trials in East Africa have indicated that the impact of STD control on HIV transmission depends on the stage of the HIV epidemic and the prevalence of STDs in a given population. The MDP study has demonstrated further that large microbicide trials can be conducted in Africa and that the acceptability of the product is high in both men and women. However there is a need to conduct more preliminary testing of microbicides, such as post-coital PD and PK studies, before large scale phase 3 trials are commenced.

Chapter 4. Important areas for future HIV prevention research

Summary

The HIV epidemic continues to pose many challenges, thirty years after AIDS was first reported. More people are living with HIV largely due to the availability of treatment but also due to continuing transmission. We have not been very successful in scaling up HIV preventive interventions especially for those at greatest risk. Over the last ten years, promising new prevention strategies have emerged but many of these have shown no effectiveness against HIV transmission and acquisition. There has, however been evidence from three recent trials in East and South Africa, which showed that male circumcision significantly reduces the risk of acquiring HIV. Building on advances in HIV treatment there is also recent evidence that antiretroviral drugs could provide prevention options through oral and topical pre-exposure prophylaxis and by using treatment to reduce the risk of HIV transmission. Though the discovery of an HIV vaccine remains elusive there are some new insights regarding where research needs to focus to advance this field.

4.1. Introduction

It is evident from data presented in the previous chapters that the HIV epidemic in Uganda continues to spread. Disappointingly, many of the interventions targeting Ugandan cohorts have had no effect on HIV transmission. Recently however, data from three trials of male medical circumcision in South Africa, Kenya and Uganda have shown approximately 60% protection among heterosexual males(33-35). A meta-analysis of results of the three trials by Weiss and colleagues estimated incidence risk ratio of 0.42 (95% CI 0.31–0.57), which is 58% (95% CI 43–69%) protective effect of circumcision(130). These data showed that male circumcision is probably the most effective biomedical HIV intervention to date. However, a comparable trial among

circumcised *HIV-infected* men showed no evidence that circumcision offers protection for their female partners(131). Despite this lack of protection for females, reduction of male HIV acquisition through circumcision does reduce women's exposure to HIV-infected men and could therefore provide an overall benefit to women.

There are various interventions that might be important for HIV prevention. Table 4.1 shows a summary of prevention methods for HIV sexual transmission, which act at different stages of the proximate determinants framework.

Table 4.1 HIV Prevention methods

Category	Intervention
Structural	Increase access to HIV prevention/clinical services
	Stigma reduction
	Supporting policies and advocacy
	Micro-finance to poor vulnerable women including "conditional cash transfers"
Behavioural interventions	HIV counselling and testing
	Condoms (male and female)
	Sex health education
	Alcohol-related risk behaviour
Biological	Treatment of STIs
	Male circumcision
	Microbicides (vaginal)
	ART for prevention
	Post exposure prophylaxis
	Oral pre-exposure prophylaxis
	Vaccines

This chapter focuses on future research on biological prevention methods that are relatively new and could potentially have a significant impact on HIV transmission. These include the role of antiretroviral drugs (ARV) for both oral and topical pre-exposure prophylaxis (PrEP) as well as HIV treatment as prevention, HIV vaccine research, and control of *Herpes simplex* virus type 2 (HSV-2). Future research priorities related to behavioural interventions are also briefly discussed.

4.2. Role of antiretroviral therapy in HIV prevention

4.2.1. Pre-exposure Prophylaxis (PrEP)

The use of ARV as pre-exposure prophylaxis in HIV prevention builds on the success of prophylaxis for other infectious diseases, for example malaria(132-134), tuberculosis(135-136) and *Pneumocystis* pneumonia(137). More direct evidence of successful prophylaxis for HIV infection has come from the effectiveness of PMTCT(138-140) and post-exposure prophylaxis (PEP)(141-142).

Over the last few years there has been considerable enthusiasm for the use of ARV in PrEP (oral and topical) for the prevention of HIV acquisition. This has been as a result of two factors. Firstly, new potent ARVs have become available to test the concept of PrEP in human clinical trials, mainly Tenofovir disoproxil fumarate (TDF) and a combination of Emtricitabine/Tenofovir (FTC/TDF) which have broad antiviral activity against both HIV viruses (HIV-1 & -2). FTC/TDF (Truvada) has favourable properties that also make it suitable for evaluation. These include its safety and tolerability profile, high drug levels in the genital tract and long intracellular half-lives, a high barrier to resistance, limited drug interactions with other drugs such as for tuberculosis and other antibiotics, easy to use on daily dosage (low pill burden) and relatively low cost due to the availability of generic formulations.

Secondly, data from PrEP studies in animal models to prevent simian immunodeficiency virus (SIV) and HIV infection have shown encouraging efficacy(143-144). Consequently, a number of PrEP clinical trials are ongoing in humans. A few have been

completed while others are in preparation (www.avac.org/trials). Briefly all these trials investigate the safety and efficacy of either TDF or FTC/TDF in various populations. Results for some of these trials have started to emerge.

For example in 2010, the iPrEx trial using FTC/TDF among MSM in several countries (USA, South Africa, Thailand, Peru, Ecuador, and Brazil) demonstrated a 44% reduction in HIV incidence (95% CI, 15%- 63%; p= 0.005). The study provided the first evidence of protection against HIV acquisition using oral PrEP. The findings also indicated that detectable blood levels strongly correlated with the prophylactic effect(38).

On the other hand, the Fem-PrEP trial that was evaluating once-daily oral Truvada versus placebo among high-risk heterosexual, HIV-negative women for HIV prevention was recently halted due to futility. A number of possible reasons have been suggested and include poor adherence and less penetration of oral Truvada in the genital tract. It has been suggested that rectal levels following oral dosing are higher than vaginal levels and this may explain the contrasting results of the iPrEx and Fem-PrEP trials(145). It has also been shown that drugs delivered topically achieve higher penetration in the female genital tract than drugs delivered through oral dosing, which may explain the difference between the results of the CAPRISA 004 and Fem-PrEP trials(146).

Encouraging as these finding are there are important questions that need to be addressed in future PrEP research. All the ongoing oral PrEP trials are evaluating daily dosing only. Are healthy HIV-uninfected recipients likely to adhere to a daily regimen for either long periods of time or for life? Successful PrEP will probably require sustained adherence. Unlike adherence to HIV treatment, little is known about the level, pattern and correlates of adherence to HIV biomedical prevention strategies such as PrEP. A better understanding of PrEP adherence behaviour will be essential to interpret clinical trial findings and maximize the effectiveness of PrEP. Are individuals likely to adhere more to an intermittent PrEP regimen? Understanding adherence to an intermittent regimen, its relationship to the timing of sexual activity and potential efficacy will be

important to evaluate. Such data will guide behavioural counselling regarding optimal use in roll out of PrEP interventions.

4.2.2. HIV treatment as prevention

The use of ARVs for the treatment of HIV has been one of the great successes in the epidemic even in countries with limited resources. Treatment initiatives have enabled greater access to treatment for many individuals resulting in improved survival and quality of life(147-149). As a means of identifying additional tools to reduce HIV transmission a new concept has emerged over recent years. That is to use ARV treatment for HIV prevention (Test and Treat). This involves universal HIV voluntary testing and treating HIV-infected individuals as early as possible with ARVs irrespective of CD4 cell count or other clinical guidelines. This could have benefits in reducing morbidity, mortality and transmission(150-151). If proved to be an effective prevention tool this could be particularly important in mature HIV epidemics in many sub-Saharan countries where most HIV transmission occurs within stable HIV discordant partnerships and where high risk behaviour plays a comparatively small role and marital partner poses the greatest risk(152).

This concept is based on evidence from a variety of sources. There are data from observational studies indicating a strong relationship between low viral load and a reduced risk of transmitting HIV to sexual partners in heterosexual populations(153). There is also evidence that reducing viral load by giving ART to the HIV-infected partner does indeed reduce transmission to a very low level(154).

Mathematical modelling has shown that a universal Test and Treat strategy may substantially reduce or even eliminate sexual transmission of HIV at a population level(155). The model made various assumptions such as high compliance to antiretroviral therapy and the availability of second-line ART regimens. In addition, the model assumes that the period of acute or primary infection, when viral load is highest and the risk of HIV transmission considered greatest, lasts only about two months and

contributes to only 10% of HIV transmissions. In reality, acute or primary infection may make a greater contribution to HIV transmission.

There is now an urgent need to establish whether this concept will work. There is additional evidence from the HPTN 052 trial that evaluated whether ARV treatment can prevent the sexual transmission of HIV among ART naïve patients with a CD4+ cell count of 350-550 cells/mm^3 in 1,750 serodiscordant couples in a number of countries (Botswana, Brazil, India, Kenya, Malawi, South Africa, Thailand and Zimbabwe). This was a two arm study in which arm 1 was randomised to ART upon enrolment plus HIV primary care, while arm 2 was randomised to HIV primary care without initiation of ART until the participant had two consecutive CD4+ cell counts within or below the range of 200-250 cells/mm^3, or developed an AIDS-defining illness. Early ART to HIV infected individuals showed a 96% reduction in the risk of HIV transmission to the HIV uninfected sexual partner (www.hptn.org).

The ultimate goal is to evaluate the impact of Test and Treat on HIV transmission at a population level by conducting a CRT using HIV incidence as an outcome measure. Such a trial would be complex and expensive. However, prior to such a commitment there are several important research questions that need to be addressed that would inform the design and conduct of such a CRT: Is universal testing and provision of ARV to all who test HIV positive feasible at a population level? What are the best and most acceptable voluntary counselling and testing strategies in different populations? How can people with acute HIV infections be identified and started on treatment? What are the attitudes towards and acceptability of such a strategy in the general population, among health policy makers, funding agencies and other stakeholders? What is likely to be the cost-effectiveness of such an intervention? These questions will need to be addressed through feasibility and smaller pilot studies preferably in different countries in order to inform the final CRT design.

4.2.3. Third generation ARV-based microbicides

The majority of new HIV infections continue to occur in women of child-bearing age through heterosexual transmission. Young women aged 15–24 years are as much as eight times more likely than young men to be HIV positive in sub-Saharan Africa(1). Among the many factors influencing female vulnerability to HIV transmission is the limited control they have over safer sexual practices such as using condoms. As discussed in chapter 3, there has been considerable interest in developing female-controlled prevention methods, in particular the development and evaluation of vaginal microbicides. The field has now moved to third generation ARV-containing products. Of these the most advanced is Tenofovir (an NRTI), available in gel form. A phase IIb trial in South Africa (CAPRISA 004), reported a 39% reduction (95% CI 6%-60%, p=0.017) in HIV infection and a 51% reduction (95% CI 22%-70%, p=0.003) in HSV2 infection among women using 1% tenofovir vaginal gel(37). The CAPRISA 004 trial design employed a coitally dependent regimen in which women were asked to apply gel up to 12 hours before sex and up to 12 hours after sex, and no more than twice in a 24 hour period (BAT24).

A second ongoing phase IIb trial, MTN003 (VOICE) is evaluating a daily dose of Tenofovir vaginal gel in several African countries and will report results in 2013. While the CAPRISA trial results offer new hope in microbicide research, confirmatory trial results are required before licensing by regulatory bodies. The current thinking is that the ongoing VOICE trial would be sufficient, if it shows effectiveness. There are however other important research questions that need to be explored which could inform wider general population use of the gel. Would less frequent use of Tenofovir gel, such as a single-dose coitally dependant regimen, be as effective and safe as the complicated two dose regimen evaluated in the CAPRISA 004 trial? The rationale for this research question is that such dosing would be simpler and more convenient, and cheaper since a smaller amount of drug would be required.

Other promising ARV-containing microbicide gel products are in development, including UC-781 and dapivirine (NNRTIs) already in phase I/II clinical trials, and

Maraviroc (CCR5 blocker) in early preclinical trials. Depending on the safety profiles these are likely to go into larger phase IIb/III clinical trials. The current promising ARV-containing microbicide products are largely being evaluated for prevention of heterosexual transmission but there is an equally urgent need to conduct rectal microbicide research. Receptive anal intercourse is a risk factor for HIV infection in both men and women. It is often difficult to obtain accurate data on the frequency of receptive anal sex in heterosexual populations although in some populations this may be significant(156-157). An effective vaginal microbicide would not offer protection to those who practice anal sex. There are many differences between the rectum and the vagina that make the rectum more vulnerable to HIV. The vaginal wall is covered by many cell layers while the rectal walls are a single cell layer thick; the rectal wall contains many more CD4 receptors making them especially vulnerable to HIV; and the rectum has a more alkaline pH which is less protective than the acidic vaginal pH. Hence evaluation of rectal microbicides is urgently required for both men and women.

Further microbicide research will be needed to explore delivery modes of the products other than a gel which is applied through an applicator and inserted in the vagina. These delivery methods include vaginal rings, film tablets and vaginal capsules. The vaginal ring impregnated with a microbicide is one approach that has already been advanced by the International Partnership for Microbicides (IPM) which is evaluating a dapivirine-impregnated vaginal ring. Though current data suggest that daily gel use is acceptable with high adherence, the vaginal ring would be inserted for several weeks providing slow release of the active product, overcoming the need for daily or coitally dependent gel insertion. Further studies on the acceptability and safety of these delivery methods will be required.

Research will also need to focus on evaluating microbicide products containing a combination of two or more ARVs either in gel or in ring form. As with HAART, use of microbicide combinations of products with different mechanisms of action could increase efficacy. For example the first combination microbicide (maraviroc and dapivirine) is already in a preclinical development phase. Whereas there are advantages

to such combination formulations, there are also potential disadvantages. There are likely to be difficulties in co-formulation, increased cost and increased potential for toxicity. All these will be important areas of research beyond the evaluation of effective single drug products.

4.3. HIV vaccine research

Much scientific effort has been put into HIV vaccine development as it is believed that this could be the best hope to end the epidemic. The overall aim is to develop a vaccine that could primarily prevent acquisition of infection and secondly control HIV progression in the event of infection. Despite the priority of vaccine development in HIV research, there has not been much success due to the challenges presented by the virus(158). These include the genetic diversity of the virus even within a single infected individual; and the ability to infect the key cells of the immune system, particularly $CD4^+$ T lymphocytes, in a relatively short time impairing the immune system in the first few weeks of infection. A successful vaccine would ideally need to meet these challenges. The few HIV vaccine trials conducted to date have generally shown no efficacy, including the STEP trial in which there was an increased risk of infection in a sub group of vaccine recipients(159). The STEP trial aimed to assess the efficacy of a cell-mediated immunity vaccine against HIV infection and change in early plasma HIV levels. The most encouraging finding in the HIV vaccine field has been the recent Thai phase III trial (RV144). This was a Phase III HIV vaccine trial that tested a "prime-boost" combination of two vaccines: ALVAC® HIV vaccine (the prime) and AIDSVAX® B/E vaccine (the boost). The trial showed that the vaccine regimen was safe and of modest benefit with an efficacy of 31.2% (95% CI 1.1-52.1). However, the vaccine regimen had no effect on viral load or CD4 cell count among volunteers who became HIV-infected during the study(36).

Despite the limited progress made in HIV vaccine research, there is evidence that provides some hope for future vaccine discovery. This is based on the observation that some HIV-infected individuals generate broadly cross-reacting neutralizing antibodies

(bNAbs). A number of these bNAbs have so far been identified (b12, 2G12, 2F5, 4E10) and more recently PG6 and PG16 isolated from a clade-A infected African patient(160). Though these bNAbs have been shown to provide sterilizing immunity in animal models(161) there have been difficulties in using them in HIV vaccine development such as constructing an immunogen that elicits the desired antibody response in animal studies. Another piece of evidence comes from "elite controllers" or natural controllers, people who are HIV-infected for several years and maintain high CD4 and CD8 cell counts, and are treatment naïve as well as having undetectable viral load(162). Further basic science research will need to focus on understanding better the role of broadly neutralizing antibodies and elite controllers in order to inform HIV vaccine design.

4.4. Control of *Herpes simplex* virus type-2

Herpes simplex virus type-2 (HSV-2) infection is a common sexually transmitted infection and is the main cause of genital ulcer disease(163-164). HSV-2 has been associated with an increased risk of HIV infection in a number of observational studies(164-165). There are a number of ways in which HSV-2 may affect HIV infection: through increased transmission, acquisition and progression. However a number of recent well conducted clinical trials have found no effect of HSV-2 suppressive therapy using acyclovir on the risk of HIV acquisition(166) or transmission(167). Despite these findings, further research may still be necessary to evaluate HSV-2 treatment for HIV control. Important questions will include assessing whether HSV-2 suppressive therapy may have a greater impact among young HSV-2 sero-converters, who on average will have been infected relatively recently and in whom HSV-2 clinical episodes are more frequent than in those who have had infection for several years. Though there is evidence that Acyclovir reduces mean plasma viral load of HIV, it is possible that a greater reduction is needed by using a higher dose and/or more potent anti-HSV-2 drugs. The finding that Tenofovir 1% vaginal gel reduced HSV-2 acquisition in the CAPRISA 004 trial is an area that will require further exploration and confirmation as this could potentially increase the impact of the gel on HIV transmission.

4.5. Future research priorities related to behaviour change interventions

Several HIV intervention trials targeting behavioural change have been conducted, including several in sub Saharan Africa. A review of HIV behavioural intervention trials indicated that none had shown a significant impact on HIV acquisition among those that used HIV incidence as outcome measure. However some showed impact on other sexual and biological indicators such as reported sexual behaviour and reduction in HSV-2 acquisition(168). Some of the possible reasons for these negative findings have included limitations of study design (sample size and duration of study), timing of interventions, and inadequacy of interventions.

Despite these findings, there are possible future research priorities for behaviour change interventions. Firstly, interventions targeting HIV positive individuals (positive prevention): most interventions have targeted HIV negative individuals and the effects of behavioural interventions targeted at HIV positive individuals need further exploration. Secondly, interventions aimed at increasing condom promotion and use need to be considered given that this is still relatively low in many developing countries despite their potential impact. Other priority areas include behavioural interventions for special groups, such as married couples, especially those in HIV discordant couple relationships; and individuals with concurrent partnerships. As most countries roll out ART programmes and other proven effective biomedical interventions, there is also a need to address issues that will help in understanding how best to avoid risk compensation in such programmes. Despite existing scientific evidence linking alcohol use with HIV sexual risk behaviour there has not been sufficient HIV prevention research in developing countries to address problem alcohol drinking(169). Interventions aimed at reducing heavy alcohol use and reducing the frequency of consuming alcohol prior to sex in conjunction with community-based efforts to reduce HIV risk behaviour have the potential to reduce the spread of HIV and this calls for investigation. Finally in countries with mature epidemics it is important to ensure that

behavioural interventions are targeted at those individuals in whom the next new infections are likely to occur, based on knowledge of local epidemics – an approach referred to as "know your epidemic" and "know your response"(170-172).

4.6. Conclusion

Though the most recent evidence of successful interventions and the focus for future HIV prevention research is on biomedical interventions there is a need to recognise that most of the biomedical interventions have strong behavioural components. The success of biomedical interventions that target both HIV transmission and acquisition will depend on understanding the impact of behavioural components such as acceptability, adherence and risk compensation. It is thus important that future HIV biomedical prevention research design should incorporate evaluation of behavioural aspects that will help in the interpretation of outcomes.

Chapter 5. Conclusion

5.1. Introduction

We are now three decades into the HIV/AIDS epidemic. HIV has had a devastating impact on the health, social and demographic characteristics of many countries especially in sub Saharan Africa(173-176). With substantial funding from a wide range of funding agencies, HIV research has been high on the agenda for many governments. This research has provided important information that has enabled us to understand the transmission dynamics, risk factors, magnitude and impact of the epidemic. Various interventions have been designed and evaluated in many settings, whose results have informed national and international policies on the management and control of the epidemic. A recent example is the policy on male circumcision, now in place in many countries following three successful circumcision trials. Despite these achievements, however, HIV remains one the greatest global health problems still being faced.

In Uganda, strenuous efforts to understand and control HIV transmission were initiated in the early years of the epidemic. These efforts together with high-level political support led Uganda to be considered one of the world's success stories in controlling HIV/AIDS. This thesis is based on ten published papers arising from research on HIV epidemiology and on HIV interventions in rural South West Uganda, conducted between 1989 and 2008. I have utilised data arising from my professional experience and the key role I played in establishing and following up unique cohorts for conducting HIV epidemiological and intervention studies. I have examined three research questions that have implications for the control of HIV transmission in African populations.

Firstly, what are the trends in HIV incidence and prevalence in Uganda? Secondly what are the key determinants of these trends? And finally what are the new strategies that could prevent HIV transmission in this population?

In chapter 1, I described the development of the MRC/UVRI research programme, the characteristics of the populations studied in the different studies, summarised the results from the ten published papers, and used a proximate-determinants framework to describe the key determinants of HIV incidence and prevalence that have been examined. In chapter 2, I critically examined trends in HIV incidence and prevalence during the study period and used the framework to explore the effects of underlying and proximate determinants on the trends observed during the study period. The limitations of the analysis, methods used are also discussed in this chapter. Chapter 3 described and critically assessed interventions to prevent HIV transmission in the study cohorts, and examined why some interventions showed no impact on transmission. Chapter 4 focused on future research on biological prevention methods that are relatively new and could potentially have a significant impact on HIV transmission. Future research priorities related to behavioural interventions are also discussed. In this final chapter I draw some conclusions arising from the work presented in the first four chapters and attempt to answer the three main questions outlined above. I begin with a note about the study populations on which my research was based.

5.2. Study populations

The cohorts in which this research was conducted were relatively stable, rural and semi-urban populations. We have been able to follow these cohorts and obtain longitudinal epidemiological data over a 20-year period through annual demographic and serological surveys with high participation rates. Of the adults enumerated at each survey round, 75.8% had a confirmed HIV test result in that round(14). These are some of the longest continuing HIV cohorts in Africa, established early in the epidemic enabling us to monitor trends in HIV incidence and prevalence in a systematic manner up to the present day.

We used similar methods of data collection, statistical analysis and laboratory testing throughout the study period. The age and sex distribution also remained the same throughout the period. Overall the total size of the population has not changed as in-

migration (including births) has been similar to out-migration (including deaths); 10.7% and 10.3% respectively. Thus the data from which the HIV trends and risk factors have been derived are robust and could be generalisable to other, similar populations elsewhere in Uganda.

To prepare for future HIV prevention research, we carefully considered the need to establish population cohorts that would be most suitable for this work. It was important to identify cohorts with high HIV incidence as this is a primary outcome measure for many HIV intervention trials. One such cohort has been described, composed of HIV negative adults living in serodiscordant stable relationships. This cohort was used successfully to recruit participants for a large phase III vaginal microbicide.

5.3. Trends in HIV prevalence and incidence in Uganda

I examined HIV prevalence and incidence trends in Uganda. Overall there was a significant decline in HIV prevalence in both sexes, especially in those aged 20-24 years between 1990 and 2000. Thereafter there was a clear reversal in trends, with HIV prevalence rising steadily until 2008. However, the trends in those aged 35 years and above have been the most discouraging – rising throughout the study period. It was not surprising that HIV prevalence was higher among females than in males, a finding that has been documented in many epidemiological studies in SSA(32, 69).

Although there are limitations in the analysis of time trends based on our cohort data, the findings from this thesis were broadly consistent with data from other sources in Uganda. Of particular interest are the data from the neighbouring Rakai Programme cohort and from the national surveillance system. Both indicated a similar decline in HIV prevalence in the 1990s but with less clear evidence of a subsequent increase in prevalence.

HIV incidence trends were more difficult to interpret because of the relatively small number of seroconversions each year. Earlier analysis up to 1999 indicated a decline in

HIV incidence in the 1990s for both men and women. Further analysis up to 2008 showed a significant decline in all ages between 1990 and 2000, and a non significant increase between 2001 and 2008. We did not observe any sex difference in incidence rates between men and women throughout the period. The observation that prevalence and incidence were already declining from the late 1980s and early 1990s when the MRC cohort was established suggests that the decline could have started a few years earlier. This has been supported by modelling of HIV incidence(57).

5.4. Key determinants of the trends

The key determinants that contributed to the decrease in HIV prevalence during the 1990s were a net outflow of HIV positive migrants, more deaths of HIV positive individuals and reductions in risky sexual behaviour, as well as the natural course of the epidemic. A number of factors could have led to these trends in sexual behaviour, including nationwide health campaigns, improved STD management and high-level political support. The increase in HIV prevalence from 2000 is more difficult to explain although improved survival due to ART, an increase in high risk sexual risk behaviour and a shift in government efforts away from HIV prevention could all have contributed. Trends in HIV incidence rates were less clear than prevalence trends. The decline in incidence seen from the early 1990s could be an indication that the decline could have started earlier, possibly in the late 1980s.

5.5. New HIV prevention strategies

Health education targeting safer sexual behaviour has been the cornerstone of HIV prevention, but current trends in HIV incidence and prevalence clearly indicate that this alone is not sufficient to control the epidemic. In an attempt to fill this gap several interventions have been evaluated. I have described two large intervention trials that evaluated the impact on HIV acquisition of improved STD management in combination with behavioural change, and a vaginal microbicide gel. Neither trial showed efficacy. Although the concepts underpinning these trials were correct, the interventions were

insufficient to demonstrate impact. Several other HIV intervention trials in other settings have also not shown efficacy against HIV transmission(98). As a consequence, there has been a call for new prevention strategies which were discussed in chapter 4. These include oral and topical pre-exposure prophylaxis using antiretroviral drugs, HIV treatment as prevention (i.e. early treatment of people with HIV to reduce their infectiousness) and HIV vaccines. There have been recent efforts to roll-out medical male circumcision (MMC) in many African countries including Uganda. It is too early to determine whether MMC together with existing behavioural approaches will be sufficient to bring the epidemic under control. The success of these biomedical interventions will to a large extent depend on behavioural factors such as acceptability, adherence and risk compensation. I recognise that there are other research priorities related to behavioural change interventions unrelated to biomedical interventions which will also require evaluation.

5.6. Further research

The concept of phase-specific dynamics of HIV epidemiology(177) suggests that the infection retreats into higher risk subgroups of the population as the epidemic matures, as is the case in Uganda. The networks in such subgroups strongly influence the risk of transmission and subsequently have important implications for interventions. There is evidence that HIV prevalence in Uganda and in other countries may be elevated in certain high risk population sub groups that deserve special attention and further research. These include individuals living in fishing communities and women engaged in sex work. It is estimated that HIV prevalence in African fishing communities is much higher (probably 3-4 times) than the national average(178-179). These high infection rates have been attributed to a number factors such as limited access to prevention and treatment, high alcohol consumption and behavioural characteristics including multiple and concurrent sexual partners as well as sexual networks reaching both within and outside the community. There have been several studies among sex workers in a number of African countries which have documented high rates of STDs and HIV infection(180-181). Some of the studies have also evaluated interventions targeted to these

groups(182-183). Intensive HIV prevention using proven effective interventions in these high risk populations is an area of high priority for Uganda.

5.7. Conclusion

The research I have conducted with my colleagues in Uganda has allowed us to monitor and understand trends in HIV incidence and prevalence. The research has also helped us understand which strategies for HIV prevention will work in this population. Hopefully, the cohorts we established in Uganda in the 1980s will continue to provide new opportunities for monitoring and preventing the transmission of HIV in this population in the future.

THE PAPERS

Paper 1

Kamali A, Carpenter LM, Whitworth JAG, Pool R, Ruberantwari A, Ojwiya A.

Seven–year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda.

AIDS 2000; 14: 427-434.

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PAGES 120-206, PREVIOUSLY PUBLISHED PAPERS

PAGES 207-220, SIGNATURES

Permissions and statements of contribution

Paper 1. Seven–year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda

Kamali A, Carpenter LM, Whitworth JAG, Pool R, Ruberantwari A, Ojwiya A

Anatoli Kamali initiated the idea for writing the paper together with Lucy Carpenter and James Whitworth. Robert Pool provided input to and interpretation of the sexual behaviour data. Anthony Ruberantwari was responsible for the data management over the study period, and Amato Ojwiya was responsible for assessment and interpretation of the HIV serology results. Anatoli Kamali supervised the data collection during the study period, and together with Lucy carpenter drafted the first draft of the manuscript. James Whitworth helped in interpretation of the results and made valuable input into the subsequent drafts of the paper up to the final version. All authors contributed to the subsequent drafts of the paper.

Anatoli Kamali | Lucy M Carpenter | James AG Whitworth

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Paper 2. Declining HIV-1 incidence and associated prevalence over 10 years in a rural population in south-west Uganda: a cohort study

Mbulaiteye SM, Mahe C, Whitworth JG, Ruberwantari A, Nakiyingi JS, Ojwiya A, Kamali A

Sam Mbulaiteye was study coordinator and oversaw field data collection between 1997and 2000, interpreted data, and wrote the first draft of the manuscript. Cedric Mahe did most of the data analysis, contributed to interpretation of data, and helped to draft the paper. James Whitworth led the protocol design, contributed to interpretation of data, and edited the paper. Anthony Ruberantwari and Jessica Nakiyingi contributed to the data management and analysis. Amato Ojwiya oversaw the laboratory data collection. Anatoli Kamali was study coordinator and oversaw field data collection between 1990 and 1997. All authors commented on and contributed to the final draft of the manuscript.

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Paper 3. HIV-1 epidemic trends in rural south-west Uganda over a 10-year period

Whitworth JAG, Mahe C, Mbulaiteye SM, Nakiyingi J, Ruberantwari A, Ojwiya A, Kamali A

James Whitworth conceived the idea of writing the paper, interpreted the data and was responsible for the initial draft through to the final draft and revisions. Cedric Mahe together with Jessica Nakiyingi did the data analysis. Sam Mbulaiteye and Anatoli Kamali were responsible for the field data collection during the periods 1997-2000 and 1990-1997 respectively and both were involved in interpretation and writing the paper to the final draft as well as revisions arising from the referee's comments. Amato Ojwiya was in charge of laboratory testing and interpretation of the HIV test results.

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James AG Whitworth

Cedric Mahe

Sam Mbulaiteye

Jessica Nakiyingi

Anthony Ruberantwari

Amato Ojwiya

Anatoli Kamali

Paper 4. Seroprevalence and incidence of genital ulcer infections in a rural Ugandan population

Kamali A, Nunn AJ, Mulder DW, Van Dyck, Dobbins JG, Whitworth JAG

Anatoli Kamali was involved in the study design, directly supervised the field data collection, was highly involved in data analysis, led the writing of the paper, and revisions based on referees comments. Andrew Nunn, was the programme statistician, and instrumental in designing the study, did most of statistical analysis and assisted greatly in writing the paper. Daan W Mulder, head of the MRC Programme at the time the study was conducted, was responsible for the overall direction of the study. He was the key person in the study design and gave tremendous support in writing the paper. Eddy Van Dyck was responsible for testing sera for *H ducreyi* and *T pallidum* in Antwerp and interpretation of test results. In addition, he contributed to the writing of the paper. Jim G Dobbins was responsible for testing sera for HSV-2 at CDC, Atlanta and interpretation of the test results. James AG Whitworth took over from Daan Mulder as the head of the programme, and was a key person in the whole process of data analysis and writing the paper from the initial draft up to the time of submission.

Anatoli Kamali Andrew J Nunn Daan W Mulder

Eddy Van Dyck James G Dobbins James AG Whitworth

Paper 4. Seroprevalence and incidence of genital ulcer infections in a rural Ugandan population

Kamali A, Nunn AJ, Mulder DW, Van Dyck, Dobbins JG, Whitworth JAG

Anatoli Kamali was involved in the study design, directly supervised the field data collection, was highly involved in data analysis, led the writing of the paper, and revisions based on referees comments. Andrew Nunn, was the programme statistician, and instrumental in designing the study, did most of statistical analysis and assisted greatly in writing the paper. Daan W Mulder, head of the MRC Programme at the time the study was conducted, was responsible for the overall direction of the study. He was the key person in the study design and gave tremendous support in writing the paper. Eddy Van Dyck was responsible for testing sera for *H ducreyi* and *T pallidum* in Antwerp and interpretation of test results. In addition, he contributed to the writing of the paper. Jim G Dobbins was responsible for testing sera for HSV-2 at CDC, Atlanta and interpretation of the test results. James AG Whitworth took over from Daan Mulder as the head of the programme, and was a key person in the whole process of data analysis and writing the paper from the initial draft up to the time of submission.

Anatoli Kamali Andrew J Nunn Daan W Mulder

Eddy Van Dyck James G Dobbins James AG Whitworth

Paper 5. HIV prevalence and incidence are no longer falling in South-Western Uganda: evidence from a Rural Population Cohort 1989 – 2005

Shafer LA, Biraro S, Nakiyingi-Miiro J, Kamali A, Ssematimba D, Ouma J, Ojwiya A, Hughes P, Van der Paal L, Whitworth J, Opio A, Grosskurth H

Leigh Anne Shafer and James Ouma conducted the statistical analysis. Leigh Anne Shafer also conducted the literature review and wrote the first draft of this paper. James Ouma and Duncan Ssematimba provided data management for the longitudinal data from the cohort from which data for this paper come. Sam Biraro was project leader of the cohort and managed day-to-day activities of data collection for the cohort. Jessica Nakiyingi-Miiro contributed to data analysis. Anatoli Kamali was the project leader who set up the cohort in 1989, designed study questionnaires and supervised the data collection throughout the 1990s. He contributed to writing all the drafts of the paper until submission. Peter Hughes and Amato Ojwiya helped to assess the HIV status when the ELISA and/or western blot test results were ambiguous. Leive Van der Paal contributed to the study design and analysis. James Whitworth and Heiner Grosskurth contributed greatly towards questionnaire development and data collection practice. Alex Opio provided an assessment of HIV results from the Ministry of Health antenatal clinics throughout Uganda which ultimately led to the development of this paper. All authors contributed to subsequent drafts of the paper until final version.

Leigh Anne Shafer	Sam Biraro	Jessica Nakiyingi-Miiro
Anatoli Kamali	Duncan Ssematimba	James Ouma
Amato Ojwiya	Peter Hughes	Leive Van der Paal
James Whitworth	Alex Opio	Heiner Grosskurth

Paper 6. A community randomized controlled trial to investigate impact of improved STD management and behavioural interventions on HIV incidence in rural Masaka, Uganda: trial design, methods and baseline findings

Kamali A, Kinsman J, Nalweyiso N, Mitchell K, Kanyesigye E, Kengeya-Kayondo JF, Carpenter LM, Nunn A, Whitworth JAG

Anatoli Kamali was the project leader from December, 1997 to 2002 when the trial ended. He supervised the implementation of the trial and delivery of the interventions, and data collection. Anatoli Kamali together with James Whitworth instigated the idea of writing the paper, and wrote the first draft. John Kinsman, Norah Nalweyiso, Kirsty Mitchell and Edward Kanyesigye were responsible for implementation of the behavioural and STD interventions. Jane Kengeya-Kayondo was involved in the study design and subsequent direction of field activities in early years of the trial. Lucy M carpenter conducted data analysis, interpretation of results and was highly involved in writing the manuscript. All authors commented on various drafts of the paper prior to submission.

Anatoli Kamali	John Kinsman	Norah Nalweyiso
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Anatoli Kamali	John Kinsman	Norah Nalweyiso
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Lucy M Carpenter	Andrew Nunn	James AG Whitworth

Paper 7. A community randomized trial of sexual behaviour and syndromic STI management interventions on HIV-1 transmission in rural Uganda

Kamali A, Quigley M, Nakiyingi J, Kinsman J, Kengeya-Kayondo J, Gopal R, Ojwiya A, Hughes P, Carpenter LM, Whitworth J

Anatoli Kamali was the project leader for the trial from December, 1997 up to the end of the trial. He supervised the implementation of the trial and delivery of the interventions, and data collection. He contributed to analysis and interpretation of the data, conducted the literature review and took the lead in drafting the paper. Maria Quigley took the lead in data analysis and contributed to its interpretation. Jessica Nakiyingi contributed to data analysis and implementation of the study. John Kinsman was responsible for the behavioural intervention and contributed to data analysis and interpretation. Jane Kengeya-Kayondo was project leader from December, 1995, to December, 1997, and contributed to initial study design, protocol develepment and implementation of the study. Robbin Gopal did the HSV2 testing. Amato Ojwiya did the HIV-1 and syphilis testing, and interpretation of results. Peter Hughes did the chlamydia and gonorrhoea testing, and interpretation of results. Lucy M Carpenter planned the statistical analysis and contributed to interpretation of data. James Whitworth contributed to design, protocol develpment and implementation of study, analysis and interpretation of data, and drafting the report. All investigators contributed to writing of the report.

Anatoli Kamali Maria Quigley Jessica Nakiyingi

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Anatoli Kamali	Maria Quigley	Jessica Nakiyingi
John Kinsman	Jane Kengeya-Kayondo	Robin Gopal
Amato Ojwiya	Peter Hughes	Lucy M Carpenter
James Whitworth		

Paper 8. The impact of attending a behaviour intervention on HIV incidence in Masaka, Uganda

Quigley AM, Kamali A, Kinsman J, Kamulegeya I, Nakiyingi-Miiro J, Kiwuwa S, Kengeya-Kayondo JF, Carpenter LM, Whitworth JAG

Maria A Quigley and Lucy Carpenter were the trial statisticians and were responsible for data analysis. Maria M Quigley initiated the idea of the sub-analysis that led to writing this paper. She also wrote the initial draft. Anatoli Kamali was responsible the overall implementation of the intervention and field data collection, and contributed generously to the writing of the paper. John Kinsman and Ignatius Kamulegeya supervised the behavioural interventions and supervision of field workers. Jessica Nakiyingi-Miiro and Sylvia Kiwuwa were in charge of data management during the trial and assisted in the data analysis. Jane Kengeya-Kayondo contributed to the study design and protocol development, and field data collection in early years. James AG Whitworth contributed to the interpretation of the data and writing the paper. All authors read and made comments on various drafts of the manuscript.

Maria A Quigley	Anatoli Kamali	John Kinsman
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Maria A Quigley	Anatoli Kamali	John Kinsman
Ignatius Kamulegeya	Jessica Nakiyingi-Miiro	Sylvia Kiwuwa
Jane F Kengeya-Kayondo	Lucy Carpenter	James AG Whitworth

Paper 9. Willingness to participate in preventive HIV vaccine trials in a community-based cohort in south west Uganda

Ruzagira E, Wandiembe S, Bufumbo L, Levin J, Price MA, Grosskurth H, Kamali A

Eugene Ruzagira was the study coordinator for HIV vaccine preparedness studies, supervised the field data collection, and wrote the first draft of the paper. Simon Wandiembe and Jonathan Levin did the data analysis and assisted with data interpretation. Leonard Bufumbo was a social science research associate and was involved in qualitative data collection, and led the community preparedness activities. Matthew A Price contributed to interpretation of data. Heiner Grosskurth contributed to editing the manuscript. Anatoli Kamali was the principal investigator for the vaccine preparedness studies, and contributed to data collection, data analysis and interpretation and writing the paper. All authors commented on various drafts of the paper.

Eugene Ruzagira Simon Wandiembe Leonard Bufumbo

Jonathan Levin Matthew A Price Heiner Grosskurth

Anatoli Kamali

Paper 10. PRO2000 vaginal gel for prevention of HIV-1 infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial

McCormack S, Ramjee G, Kamali A, Rees H, Crook AM, Gafos M, Jentsch U, Pool R, Chisembele M, Kapiga S, Mutemwa R, Vallely A, Palanee T, Sookrajh Y, Lacey CJ, Darbyshire J, Grosskurth H, Profy A, Nunn A, Hayes R, Weber J:

Sheena McCormack was the chief investigator for the trial and prepared the first draft of the paper. Gita Ramjee, Anatoli Kamali, Helen Rees, Mitzy Gafos, Maureen Chisembele, Saidi Kapiga were the country principal investigators who oversaw the day-to-day conduct of the trial and onsite data management. Angela M Crook did the statistical analysis and the initial draft of the tables. All authors contributed to study design and conduct and revision of the final draft of the paper.

Sheena McCormack	Gita Ramjee	Anatoli Kamali
Helen Rees	Angela Crook	Heiner Grosskurth
Andrew Nunn	Richard Hayes	Jonathan Weber

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